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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00012-32 BRB

PERIOD COVERED

October 1, 1993 - September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Infrared and Raman Spectroscopy of Teeth, Bones and Related Synthetic Compounds

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

B.O. Fowler, Research Chemist, BRB, NIDR

COOPERATING UNITS (if any)

ADAHF, NIST, Gaithersburg, MD; NIST, Gaithersburg, MD

LAB/BRANCH

Bone Research Branch

SECTION

Mineral Chemistry and Structure Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.00

PROFESSIONAL:

1.00

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The main objective is to determine compositional and structural details of the inorganic phase in teeth and bones. Infrared and Raman spectroscopy, x-ray diffraction and chemical methods are employed in these studies. Methods are devised for the preparation of synthetic calcium apatites having controlled physical properties (crystal size and perfection) and chemical constituents (hydroxide, fluoride, chloride, carbonate, water, acid phosphate and other ions). The vibrational spectra of these apatites and related compounds are assigned and characterized. Isotopically enriched apatite analogs are prepared to facilitate spectral assignments. The spectroscopic assignments and supplemental spectral data (temperature dependence and polarization) are then utilized to establish compositional and structural details of the apatites in question, which include: the type and geometry of constituent ions; the site or number of sites occupied by the ions; orientation of ions; chemical bonding and interactions of ions; and semi-quantitative estimations of the constituents present. The results for these controlled apatite systems are then related to the inorganic phase in calcified tissues. Infrared and Raman band assignments were made for the biologically important compound, octacalcium phosphate. Specific assignments were made for the two crystallographically different acidic phosphate groups, and infrared spectra indicate two polymorphs of octacalcium phosphate may exist. The formation of octacalcium phosphate carboxylates in solution from 19 different carboxylates was investigated; 10 octacalcium phosphate carboxylates were formed. Detailed characterization (infrared, Raman, x-ray diffraction and chemical) of six of these octacalcium phosphate carboxylates containing structurally incorporated succinate, adipate, suberate, sebacate, fumarate and citrate ions was carried out. By utilizing the combined data, the possible positions of dicarboxylate ions in the octacalcium phosphate carboxylate structures were inferred. The solubility product of octacalcium phosphate succinate was determined.







DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00074-22 BRB

PERIOD COVERED

October 1, 1993 - September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Bone and Tooth Matrix Biochemistry and Metabolism

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

L.W. Fisher, Research Chemist, BRB, NIDR  
J.C. Woodard, Special Volunteer, BRB, NIDR  
J.T. Stubbs, IRTA Fellow, BRB, NIDR  
H. Green, Biological Aide, BRB, NIDR

COOPERATING UNITS (if any)

Sackler School of Medicine, Tel Aviv, Israel; Universita "La Sapienza", Rome, Italy;  
Dental Research Unit, Hebrew University, Jerusalem, Israel

LAB/BRANCH

Bone Research Branch

SECTION

Skeletal Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.55

PROFESSIONAL:

2.0

OTHER:

.55

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

For FY 1993 the Protein Biochemistry Group was involved in six major projects each involving the skeletal extracellular matrix and/or the interaction of cells with matrix. 1) In collaboration with our colleagues, we have further refined the chromosomal localizations of two matrix gene products likely to be important in oral development, tuftelin and bone sialoprotein (BSP). A useful nucleotide repeat polymorphism for BSP has been identified for humans and a candidate repeat sequence has been found for the tuftelin gene. 2) Using a monoclonal antibody that detects a marker specific for a particular stage of marrow stromal cell development along the osteoblastic lineage, we have cloned and sequenced a unique 5 kbp cDNA. 3) We have screened a series of cell lines looking for one that divides rapidly but that also maintains biglycan (BGN) proteoglycan on its cell surface as well as the slower growing normal human bone cells. Our working hypothesis is that the BGN propeptide retains the proteoglycan on cell surface but to date we have been unsuccessful at finding a useful cell line. 4) We have made recombinant versions of three leucine-rich repeat proteins. One of them, a fragment of the acid-labile subunit of the human IGF-binding protein, was made incorporating N15 and C13 stable isotopes for use in NMR structural analysis. Using conditions tried to date, the protein self associates into a complex too large to solve by NMR (with Dr. Dennis Torchia). 5) Recombinant fragments of human BSP have been successfully made to study: a) the cell attachment to the RGD domain as well as mutation analogs; b) the structure of the sixty amino acid RGD domain by NMR (a random coil); c) cell attachment to deletion mutations to determine the sequence of the second, cryptic cell attachment domain; and d) deletion mutations to determine the hydroxyapatite-binding domain(s) (with Dr. David Eanes). 6) Dr. J. Carroll Woodard has joined the group for three months this summer to begin histological, immunohistological and *in situ* studies on the early events in the vitamin A-induced premature growth plate closure in experimental calves.







DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00088-21 BRB

PERIOD COVERED

October 1, 1993 - September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Chemical, Structural and Morphological Studies on Calcium Phosphates

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

E.D. Eanes, Chief, MCSS, BRB, NIDR  
D. Skrtic, Visiting Associate, BRB, NIDR  
A.W. Hailer, Chemist, BRB, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Bone Research Branch

SECTION

Mineral Chemistry and Structure

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.5

PROFESSIONAL:

1.0

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The purpose of this project is to study the physical, chemical, and ultrastructural properties of calcium phosphate salts, and to clarify the kinetic and thermodynamic processes and the interactions with substances of biological interest that uniquely enable calcium phosphate salts to carry out their specialized role in vivo. The properties of calcium phosphate salts are being studied with a variety of ultrastructural and physical-chemical techniques such as electron microscopy, x-ray diffraction, surface area analyses, chromatographic and standard analytical chemistry procedures. The principal endeavor currently being pursued involves artificial lipid vesicles (liposomes) as in vitro models to investigate physico-chemical aspects of matrix vesicle (MV)-mediated calcification in vivo. The latest phase of this endeavor is a study that is examining the effect that organic phosphonates have on mineral development in the liposomal model system. The aim of this study is to better delineate the physicochemical basis for the observed suppressive effects bisphosphonates such as 1-hydroxyethylidene-1, 1-bisphosphonate (HEBP) have on MV calcification. Findings from this study showed that HEBP had little, if any, effect on precipitation initiated within the aqueous cores of the liposomes but had a significant, dose-dependent, negative impact on the subsequent spread of the precipitation into the surrounding extraliposomal medium. The inhibitory effect of HEBP was more strongly felt in suspensions of liposomes in which phosphatidylserine (PS) was a membrane component. HEBP also was more effective when added to the suspension medium rather than encapsulated within the liposomes themselves. The data suggest that direct binding to growth sites on mineral surfaces blocking further accretion from solution most likely accounts for HEBP's inhibitory effect. The results could also explain the finding (Morris et al., Bone 1990;11:281) that HEBP in vivo suppresses more effectively extravesicular calcification than precipitation within the MVs themselves.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00379-11 BRB

PERIOD COVERED

October 1, 1993 - September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Bone Matrix Gene Expression

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

M.F. Young, Research Biologist, BRB, NIDR

J.M. Kerr, Staff Fellow, BRB, NIDR

K. O'Connor, Visiting Associate, BRB, NIDR

T. Xu, IRTA Fellow, BRB, NIDR

P. Dominguez, Guest Researcher, BRB, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Bone Research Branch

SECTION

Skeletal Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

3.91

PROFESSIONAL:

3.91

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The matrix proteins of bones and teeth play key roles in the structure and function of these tissues. Our objective is to study the structure and function of these macromolecules and to understand the regulation of their expression. The primary structures of bone and tooth matrix proteins have been studied by constructing recombinant cDNA libraries from bone or ameloblast cell mRNA. cDNAs encoding several bone and tooth matrix proteins were isolated. The clones and antibodies were used to determine the primary structure and mode of expression of the genes in cultured cells and intact tissue. The deduced amino acid sequence of mouse and bovine bone sialoprotein showed that the protein is remarkably conserved particularly in potentially functional domains such as the highly acidic amino acid terminus and the gly-arg-asg sequence that is presumed to bind integrin receptors in bone cells. The chromosome location of BSP gene (IBSP) was mapped in the mouse and shown to be on chromosome 5 between the Pdeb and mgas loci. The entire coding region of the human IBSP gene was isolated and the genomic organization and potential regulatory elements elucidated. Studies on osteonectin gene regulation indicate that a novel purine binding protein may be involved in tissue specific transcription of the gene and has a molecular weight of ~40,000 kd. Studies are underway using transgenic mice to identify the function of the matrix proteins and the elements that regulate their expression during development and aging in vivo.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 DE 00380-11 BRB

PERIOD COVERED

October 1, 1993 - September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Metabolism of Bone Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

P. Gehron Robey, Biologist, BRB, NIDR  
W.J. Grzesik, Visiting Associate, BRB, NIDR  
A.J. Friedenstein, Special Volunteer, BRB, NIDR  
C. Crescioli, Visiting Fellow, BRB, NIDR  
S. Kuznetsov, Visiting Associate, BRB, NIDR  
M. Atkinson, Biological Aide, BRB, NIDR  
D.W. Rowe, IPA, BRB, NIDR

COOPERATING UNITS (if any)

Department of Biopathology, Universita "La Sapienza", Rome, Italy

LAB/BRANCH

Bone Research Branch

SECTION

Skeletal Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

5.75

PROFESSIONAL:

5.0

OTHER:

.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The primary goals of the Cellular Biochemistry Group are to determine the composition and functional features of the supramolecular complex of proteins that calcifies, and how cells regulate this process. Towards these aims, cell cultures that form mineralized tissues were established for biochemical analysis, and for studies at the genomic level in collaboration with Drs. Marian F. Young and Larry W. Fisher. Comparison of normal, non-transformed cell lines to osteosarcoma cell lines revealed that the osteosarcoma cells differ dramatically in the bone matrix protein profile from normal cells, and from what is expressed in vivo. A major difference is the co-expression of early and late markers of matrix mineralization. Characterization of bone protein production in vitro and in vivo continued. It was found that while there are six RGD-containing proteins made by bone cells (thrombospondin, fibronectin, vitronectin, collagen, osteopontin and bone sialoprotein), each displays a different pattern in vivo, and exhibits different cell attachment properties in vitro. It was also found that the pattern of integrin expression changes with stage of maturation, and in particular, the  $\alpha_4$  subunit of the fibronectin receptor is dramatically increased in the cell layer. A preosteoclastic cell line, FLG 29.1, was found to produce BSP after stimulation with TPA, a property also noted in osteoclasts in vivo. A new antibody against the propeptide of osteocalcin indicated that osteocytes, rather than osteoblasts, are the major source of osteocalcin in human subperiosteal bone, and studies in vitro indicated that 1,25-dihydroxy vitamin D<sub>3</sub> induced cleavage and secretion of the precursor molecule. Lastly, studies utilizing bone-forming cell cultures indicated that there are major changes in rate of proliferation and secretion of the non-collagenous proteins, irrespective of the detectability of a collagen mutation. These changes in matrix composition may contribute significantly to the pathophysiology of the disease.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00507-05 BRB

PERIOD COVERED

October 1, 1993 - September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

NMR Structural Studies of TGF- $\beta$ 1 and HIV-1 Protease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

D.A. Torchia, Chief, PBS, BRB, NIDR

S. Archer, Guest Researcher, BRB, NIDR

A.P. Hinck, IRTA Fellow, BRB, NIDR

L.K. Nicholson, IRTA Fellow, BRB, NIDR

T. Yamazaki, Visiting Associate, BRB, NIDR

COOPERATING UNITS (if any)

LCP, NCI; Celtrix Laboratories; R&D Systems

LAB/BRANCH

Bone Research Branch

SECTION

Protein Biophysics Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

3.58

PROFESSIONAL:

3.58

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

NMR structural studies of two HIV related proteins are underway, the HIV-1 protease and TGF- $\beta$ 1.

(1) HIV-1 protease. The HIV protease is a leading candidate for targeted antiviral drug design because inhibition of this enzyme results in production of non-infectious virions. NMR signal assignments have been obtained for two protease inhibitor complexes, and work is nearly complete on determining the high resolution 3D structure of the protease complexed with DMP323, a cyclic urea based protease inhibitor, having high affinity, specificity and bioavailability. We have also shown that the urea oxygen of the DMP 323 inhibitor replaces a structural water molecule found in the structure of all reported HIV protease-inhibitor complexes.

The molecular dynamics of the protease in the presence of both DMP323 and a linear inhibitor P9941, have been compared using  $^{15}\text{N}$  spin relaxation measurements.

Finally we have determined the pKa values of all Asp and Glu sidechains in the protease-DMP323 complex, including the Asp25,25' residues essential for activity.

(2) TGF- $\beta$ 1 structure. TGF- $\beta$ 1 up- and down-regulates proliferation of HIV infected cells. We have shown that the secondary structure of the protein in solution is in agreement with an independently determined X-ray structure of TGF- $\beta$ 2, except in a domain that distinguishes the activities of the  $\beta$ 1 and  $\beta$ 2 isoforms. Using NOE data recently derived from a partially but strategically  $^{15}\text{N}/^{13}\text{C}$  enriched protein sample, we are determining the high resolution 3D structure of TGF- $\beta$ 1 in solution. Our goal is to make a detailed comparison of the 3D structure of the two isoforms in order to derive a basis for understanding differences in their function. The significance of this project arises from the unique, detailed structural information that is being obtained about HIV related proteins in solution. This information will form the basis for rational drug design based upon the understanding of the function of these proteins in terms of interactions at the molecular level.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00510-05 BRB

PERIOD COVERED

October 1, 1993 - September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Cartilage Matrix Metabolism

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

T.I. Morales, Expert, BRB, NIDR

P.E. Long, Biologist, BRB, NIDR

M. Montgomery, Biological Aide, BRB, NIDR

COOPERATING UNITS (if any)

Laboratory of Chemoprevention, NCI, NIH

LAB/BRANCH

Bone Research Branch

SECTION

Proteoglycan Chemistry Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.6

PROFESSIONAL:

1.0

OTHER:

0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The long term objective of this program is to elucidate the intrinsic regulatory mechanisms that control the structure and function of the resilient layer of articular cartilage that covers and protects the ends of bone. We showed that TGF- $\beta$  has the ability to prevent the spontaneous proteoglycan loss that occurs in basal cartilage organ cultures by increasing biosynthetic rates and decreasing degradation. We proposed that TGF- $\beta$  is an intrinsic modulator of cartilage proteoglycan metabolism and obtained a line of evidence to support this hypothesis. For example, we showed that TGF- $\beta$  is a strong antagonist of the most powerful known resorptive agent for cartilage, retinoic acid. This vitamin increases catabolism and depresses rates of re-deposition. In the original experiments, the two signaling factors were added together to the cartilage explants. We have now modified our protocols to ask whether TGF- $\beta$  is an effective repair agent following retinoic acid treatment. After a week of treatment with retinoic acid, proteoglycan synthesis falls to <10% of controls and recovery following removal of the retinoid from the medium is inadequate (an average 2-fold increase over retinoid treated samples). Recovery is greatly improved by TGF- $\beta$  (10-20 fold increase). However, the rates of synthesis in the presence of TGF- $\beta$  following retinoid exposure generally do not reach the levels of control cultures treated with the cytokine. The effect of IGF-1 mirrors that of TGF- $\beta$ . However, when both TGF- $\beta$  and IGF-1 are added to the recovering cultures, the anabolic rates rise to levels comparable to those of similarly treated control cultures. Similarly, catabolic rates, which are increased ~4-5 fold by retinoic acid treatment are returned to control levels by treatment with TGF- $\beta$  and IGF-1. The net result of recovery in the presence of the cytokines is a stabilization of proteoglycan levels existing at the time of switching. This provides further support for the role of TGF- $\beta$  and IGF-1 in the control of cartilage metabolism.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 DE 00548-03 BRB

PERIOD COVERED

October 1, 1993 - September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Study of Proteoglycan Biosynthesis in the Golgi Apparatus Using Brefeldin A

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

Masaki Yanagishita, Visiting Scientist, BRB, NIDR

Anthony Calabro, Staff Fellow, BRB, NIDR

Vincent Hascall, Guest Researcher, BRB, NIDR

COOPERATING UNITS (if any)

University of Tromso, Norway

LAB/BRANCH

Bone Research Branch

SECTION

Proteoglycan Chemistry Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.37

PROFESSIONAL:

1.37

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The glycosaminoglycan components of proteoglycans are biosynthesized and modified in the golgi apparatus by highly organized carbohydrate transfer enzymes and sulfotransferases. The purpose of this project is to investigate the functional organization and subcellular localization of these enzyme complexes. Brefeldin A (BFA) is a chemical which specifically blocks anterograde protein transport within the golgi apparatus. It was used to disrupt the normal biosynthetic processes for adding glycosaminoglycan chains onto proteoglycans. We examined the effects of BFA on the synthesis of hyaluronan (HA) and aggrecan, two of the major extracellular matrix molecules, in rat chondrosarcoma cells. Biosynthesis of chondroitin sulfate (CS) associated with aggrecan was rapidly inhibited to >1% of the control, while that of HA continued at the normal level. This result was consistent with the current model that the biosynthesis of CS requires the transport of the core protein through the Golgi apparatus, while that of HA occurs at the plasma membrane and therefore is independent of the vesicular transport. When ovarian granulosa cells were treated with BFA, dermatan sulfate proteoglycan synthesis was abolished whereas heparan sulfate proteoglycan synthesis was only partially inhibited, suggesting that dermatan sulfate and heparan sulfate assembly on proteoglycans occurs in different subcellular compartments. The finding that only normal heparan sulfate protein core proteins were substituted with heparan sulfate chains in the presence of the drug indicated that glycosylation enzymes are highly specific to core proteins. Topics of present interest include elucidation of core protein structure which determines highly specific glycosylation enzymes.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00549-03 BRB

PERIOD COVERED

October 1, 1993 - September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Metabolism of Cell Surface Heparan Sulfate Proteoglycans

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

Masaki Yanagishita, Visiting Scientist, BRB, NIDR

Duncan Hiscock, Visiting Fellow, BRB, NIDR

Chee Keng Ng, Visiting Fellow, BRB, NIDR

COOPERATING UNITS (if any)

Department of Microbiology and Immunology, University of Michigan; Division of Cytokine Biology, CBER, FDA

LAB/BRANCH

Bone Research Branch

SECTION

Proteoglycan Chemistry Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.50

PROFESSIONAL:

2.50

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Cell surface heparan sulfate proteoglycans are widely distributed throughout animal tissues, and are involved in critical cell functions such as cell-cell and cell-extracellular matrix interactions. Their interaction with a variety of molecules including growth factors, viruses, and extracellular matrix proteins, have important biological functions. The purpose of this project is to study the metabolism of cell surface heparan sulfate proteoglycans with focus on mechanisms involved in their endocytosis and subsequent intracellular processing. We have studied intracellular localization of heparan sulfate proteoglycan (HSPG) and dermatan sulfate (DS) PGs in an osteoblastic cell line (UMR 106) using metabolic radiolabeling experiments in combination with subcellular fractionation techniques and examination with electron microscopy. Results indicated that a large proportion of HSPGs formerly identified in nuclear fractions are contamination from plasma membranes and that the small DSPG (probably biglycan and/or decorin) which associates with the cell surface may well traffic through the nucleus in these cells. Another series of experiments revealed that the source of sulfur in the intracellular pool of PAPS, a critical sulfate donor in proteoglycan sulfation, was mainly derived from environmental sulfate but not from sulfur containing amino acids. We also studied biosynthesis of bone sialoprotein (BSP) by a human osteoclastic cell line (FLG 29.1) during its differentiation induced by TPA using metabolic radiolabeling experiments. Topics of present interest include: (1) characterization of the intracellular trafficking and processing pathway for the dermatan sulfate proteoglycan which may enter the nuclear compartment; (2) further development of the procedure to isolate quantitatively and purify nuclei from UMR 106 osteoblastic cells and granulosa cells; and (3) define the intracellular subcompartments where the intercalated heparan sulfate proteoglycans are selectively degraded; and (4) study functional roles of cell surface heparan sulfate proteoglycans in infection processes by human immunodeficiency virus and herpes simplex virus-I.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 DE 00550-03 BRB

PERIOD COVERED

October 1, 1993 - September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biosynthesis and Extracellular Matrix Organization of Hyaluronic Acid

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

Masaki Yanagishita, Visiting Scientist, BRB, NIDR

Antonella Camaioni, Visiting Fellow, BRB, NIDR

Juan Carlos Calvo, Visiting Scientist, BRB, NIDR

Vincent Hascall, Guest Researcher, BRB, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Bone Research Branch

SECTION

Proteoglycan Chemistry Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.61

PROFESSIONAL:

1.61

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Hyaluronic acid (HA) is a unique glycosaminoglycan. Unlike others, which are synthesized on core proteins in the Golgi to form proteoglycans, HA is not assembled on a core protein. Rather, it is synthesized at sites associated with the plasma membrane with the elongating chain being extended into the extracellular matrix. The biological functions of HA in the extracellular matrix are based on the ability of the HA molecules to occupy large hydrodynamic domains and to interact with various specific proteins which constitute structural components in the matrix or are associated with the cell surface. The purpose of this project is to study the organization and biological roles of HA in the extracellular matrix in model cell culture systems. We have studied formation of a HA-rich extracellular matrix by cumulus cell-oocyte complex (COC) isolated from mouse ovarian follicles and the role of a serum factor which organizes HA into matrix form. Biosynthesis of HA by cumulus cells was dependent on the presence of follicle stimulating hormone and a TGF- $\beta$ -like growth factor produced by the oocyte. In addition, maximum retention of HA in the COC matrix occurred only when serum (1%) was continuously present, suggesting that the serum factor, identified as an inter- $\alpha$ -trypsin inhibitor was a structural component of the matrix. Addition of exogenous HA or of HA oligomers of decasaccharide size or larger effectively displaced endogenously synthesized HA from the matrix into the medium, while other glycosaminoglycans did not, indicating the specificity of interactions between HA and the serum factor. Topics of current interest include: (1) determination of the mechanism by which newly synthesized HA is organized into a highly viscoelastic network during differentiation of 3T3 L1 cells into adipocytes and (2) identification of HA binding proteins which are necessary in forming HA network in the extracellular matrix.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00552-03 BRB

PERIOD COVERED

October 1, 1993 - September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Differentiation Factors In Cartilage and Bone Formation and Regeneration

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

F.P. Luyten, Visiting Scientist, BRB, NIDR  
S. Vukicevic, Visiting Scientist, BRB, NIDR  
P. Chen, Visiting Associate, BRB, NIDR  
B. Hoang, Special Volunteer, BRB, NIDR  
S. Tomaski, Guest Researcher, BRB, NIDR

COOPERATING UNITS (if any)

School of Medicine, Zagreb, Croatia; Johns Hopkins, Baltimore, MD; Endocrinology and Reproduction Branch, NICHD; Creative BioMolecules, MA

LAB/BRANCH

Bone Research Branch

SECTION

Bone Cell Biology Group

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

3.25

PROFESSIONAL:

1.92

OTHER:

1.33

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objectives of this project are to study the cartilage and bone inducing factors and to define their role in embryogenesis and in postnatal life, both in tissue formation and in disease. As tissue regeneration recapitulates the developmental sequence of embryonic tissue formation, it is conceivable that understanding the mechanisms of action of the soluble differentiation factors is a key step towards biologically controlled regeneration of skeletal tissues. This will have a significant impact on the treatment of congenital and/or acquired skeletal diseases such as large bone defects, impaired fracture healing, osteoarthritis, osteoporosis and periodontitis. This project focuses on the further characterization of cartilage and bone inducing molecules, members of the TGF- $\beta$  superfamily, and their biological activities. Using molecular probes, we are studying their respective contributions to cartilage and endochondral and membranous bone formation. Immunohistochemical localization and in situ hybridization of cartilage and bone inducing proteins, as well as studies *in vitro*, indicate the selective contribution of these molecules to initiation, enhancement, maintenance and maturation of the chondrocytic and osteoblastic phenotypes.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00554-03 BRB

PERIOD COVERED

October 1, 1993 - September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Physicochemical Studies on Calcium Phosphate Cements

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

E.D. Eanes, Chief, MCSS, BRB, NIDR

COOPERATING UNITS (if any)

Dental and Medical Materials Group, Polymers Division, NIST, Gaithersburg, MD;  
ADAHF Paffenbarger Research Center, NIST, Gaithersburg, MD; Tokushima University,  
Japan

LAB/BRANCH

Bone Research Branch

SECTION

Mineral Chemistry and Structure

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.1

PROFESSIONAL:

.1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

Self-setting calcium phosphate cements (CPC) are promising materials which have a variety of possible medical and dental applications. In situ setting and biocompatibility properties make CPCs potentially useful as endodontic filling materials, as implants for bony defects, and as a binder for other implant materials. CPCs are formed by moistening biphasic mixtures of calcium phosphate salts, usually anhydrous dicalcium phosphate (DCPA) and tetracalcium phosphate (TTCP), with limited amounts of water. Although relatively simple materials in composition, other chemical as well as physical properties, e.g. setting times, porosity and strength, are dependent in a complex manner upon a number of poorly understood parameters associated with the chemistry of the setting process. Particularly relevant are the solution parameters important in establishing the crystalline texture (i.e., size, shape, and aggregation properties) of the apatitic product formed upon completion of the DCPA/TTCP conversion, since the texture of this phase is a major determinant of the mechanical behavior of these cements. Thus, studies of solution influences on apatite crystal growth may prove useful in formulating cements with improved mechanical properties. In this project, the effect of solution supersaturation on apatite growth at pH 7.4 and 37°C was examined. Results suggest that growth of individual apatite crystals was superseded by crystal proliferation as supersaturation increased. This transition from primary growth to secondary nucleation results in a more finely textured apatitic product.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 DE 00574-02 BRB

PERIOD COVERED

October 1, 1993 - September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Amorphous Calcium Phosphate as an Inorganic Component in Dental Materials

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

D. Skrtic, Visiting Associate, BRB, NIDR

E.D. Eanes, Chief, MCSS, BRB, NIDR

A.W. Hailer, Chemist, BRB, NIDR

COOPERATING UNITS (if any)

Dental and Medical Materials Group, Polymers Division, NIST

LAB/BRANCH

Bone Research Branch

SECTION

Mineral Chemistry and Structure

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.4

PROFESSIONAL:

0.9

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In this project, amorphous calcium phosphate (ACP), an important intermediate in the formation of apatite, is being investigated for possible use as a dental material. When either used alone, or in combination with other dental materials, especially polymeric resins, ACP has a wide range of possible applications such as in restorative composites, cavity liners and bases, luting and pulp capping agents, prophylactic and endodontic sealants, and as a component in periodontic packs and impression pastes. It has a number of potential advantages over other calcium phosphates for these purposes. As a dental cement, its advantage over current biphasic systems (e.g., dicalcium/tetracalcium phosphate mixtures) is its simpler, single solid phase formulation. When included as a component in appropriate resin-based composites, sealants and adhesives, ACP may be useful as a remineralization agent as well as a vehicle for sustained, controlled release of inorganic anticaries ions such as fluoride. In this regard, chemical studies on various ACP-resin formulations indicate that ACP-embedded, methacrylate resins release calcium and phosphate ions at levels that exceed the thermodynamic minimum necessary for remineralizing damaged tooth surfaces. Currently, in vitro studies on bovine teeth are being carried out to evaluate the suitability of ACP-resin composites as remineralizing dental sealants. Results to date indicate that in artificial saliva-like test solutions, significantly higher levels of remineralization occur in tooth lesions coated with ACP-containing resins compared to unfilled or apatite-containing resins.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00575-02 BRB

PERIOD COVERED

October 1, 1993 - September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

NMR Structural Studies of Profilin

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

D.A. Torchia, Chief, PBS, BRB, NIDR

S. Archer, Guest Researcher, BRB, NIDR

COOPERATING UNITS (if any)

Johns Hopkins University Medical School

LAB/BRANCH

Bone Research Branch

SECTION

Protein Biophysics Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.53

PROFESSIONAL:

.53

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Acanthamoeba profilin-1 is a 125 residue protein that binds to actin, to the head groups of poly-phosphoinositides and to proline rich oligopeptides, and is thought to link regulation of actin assembly with the phosphoinositide signaling pathway. We have obtained the first three dimensional structure of profilin using multi-dimensional heteronuclear NMR spectroscopy. The central feature of the profilin solution structure is a five stranded antiparallel beta sheet flanked by N- and C-terminal helices on one face, and by a third helix and a two stranded antiparallel beta sheet on the other face. Cross linking experiments and the location of conserved residues in profilins in different phyla suggest that actin binding occurs on the face of the protein occupied by the terminal helices. The binding of actin to the C-terminal helix has been confirmed by a recent X-ray structure of the actin profilin complex. Recently we have shown that the region between the terminal helices contains a deca-L-proline binding site that is made up of several conserved aromatic residues. This region of the profilin structure is homologous to a region of the SH3 domain of tyrosine kinases that bind to proline rich peptides. The significance of the project is that the profilin structure obtained provides the first information about the interactions of profilins with actin, lipids and proline rich peptides. These interactions are thought to link processes that regulate transmembrane signaling with formation of the cytoskeleton.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00576-02 BRB

PERIOD COVERED

October 1, 1993 - September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

NMR Structural Studies of Fibronectin Modules

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

D.A. Torchia, Chief, PBS, BRB, NIDR

V. Copié, IRTA Fellow, BRB, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Bone Research Branch

SECTION

Protein Biophysics Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.55

PROFESSIONAL:

1.55

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Fibronectin is a multidomain protein with a myriad of functions. Our goal is to determine the three dimensional structure of the domain consisting of the ninth and tenth type III modules. This domain contains the RGD cell attachment sequence and has full fibronectin binding activity. In order to obtain structures using NMR methods, it is necessary to work at protein concentrations of ca. 1mM. Although the human fibronectin domain is soluble at this concentration, it aggregates under all conditions of pH, ionic strength and temperature that we have employed, and gives poor spectra at concentrations of greater than 0.2mM. Recently, Dr. Akiyama has made the recombinant mouse protein which yields good spectra at concentrations of up to ca. 0.8mM. Using this sample protein, signals have been assigned and the structure determination is underway. The significance of the project is the insight that the structure of the RGD fibronectin domain will provide about cell attachment and integrin recognition.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00603-02 BRB

PERIOD COVERED

October 1, 1993 - September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Bone Morphogenetic Proteins: Biological Significance of Their Redundancy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

S. Vukicevic, Visiting Scientist, BRB, NIDR

F.P. Luyten, Visiting Scientist, BRB, NIDR

P. Chen, Visiting Associate, BRB, NIDR

COOPERATING UNITS (if any)

School of Medicine, Zagreb, Croatia; ACTA, Faculty of Dentistry, Holland; Creative BioMolecules, Hopkinton, MA

LAB/BRANCH

Bone Research Branch

SECTION

Bone Cell Biology Group

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.75

PROFESSIONAL:

.75

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The objective of this project is to define the role of bone morphogenetic proteins in cartilage and bone formation, both in embryogenesis and in postnatal life. Localization studies of BMP-3 and BMP-7 showed a specific pattern of coordinate expression during the development of several organs, in particular bone, tooth, kidney and lung, which were also the major sites of their synthesis. This suggested the broader physiological functions for BMPs. The finding that BMP-7 protein was localized to basement membranes of organs, which did not show evidence of local synthesis, suggested a systemic role for BMP-7. To test their involvement in the development of kidney which is also directly involved in the metabolism of bone, we tested the function of BMP-3, 4 and 7 in the induction of metanephric mesenchyme and branching of the ureteric bud. Preliminary data indicate that BMP-3 induces condensation of the mesenchyme to form glomeruli, which in turn induces ureteric bud to differentiate into the secretory kidney structures. In parallel studies osteopenic and rachitic animals were injected with soluble BMP-7 and it was found that BMP-7 increased the bone volume in retired breeders and ovariectomized rats. In rachitic animals it reversed the osteomalacia-associated impairment of mineralization of calcified cartilage and trabecular bone comparable to rachitic animals that received vitamin D. Future experiments should provide evidence whether kidney secretes BMPs systemically and how they effect bone metabolism coupling bone formation to resorption.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 DE 00611-01 BRB

PERIOD COVERED

October 1, 1993 - September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Parathyroid Cell Functions by Extracellular Calcium

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

Kazushige Sakaguchi, Visiting Scientist, BRB, NIDR

Masaki Yanagishita, Visiting Scientist, BRB, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Bone Research Branch

SECTION

Proteoglycan Chemistry Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.85

PROFESSIONAL:

0.85

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The parathyroid gland is the major endocrine organ which regulates extracellular calcium by secreting parathyroid hormone (PTH). Extracellular calcium is not only vital to fundamental cell functions but also plays pivotal roles in the metabolism of skeletal tissues. The feed back mechanism of calcium regulation by PTH involves sensing mechanisms of extracellular calcium by parathyroid cells and the calcium mobilizing action of PTH at peripheral tissues. The main purpose of this program is to study (1) how parathyroid cells sense extracellular calcium and (2) how this signal regulates parathyroid cell functions.

We have established a unique parathyroid cell line which retains its physiological functions in culture conditions. Decreased levels of extracellular calcium induce the expression and secretion of PTH-related peptide and cell proliferation. Parathyroid cell growth regulation by extracellular calcium has been demonstrated to be highly dependent on an autocrine system involving acidic fibroblast growth factor (aFGF) and its cell surface receptor (aFGF-R). The topic of current interest is to study effects of extracellular calcium on the expression and posttranslational modification of aFGF and aFGF-R by parathyroid cells.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00212-18 CI

PERIOD COVERED

October 1, 1993 - September 30, 1994

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Taste and its Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Weiffenbach, James	Research Psychologist	CIPC	NIDR
Fox, Philip C.	Dental Officer/Chief, CI	CIPC	NIDR
Ryba, Nicholas	Visiting Associate	LI	NIDR

COOPERATING UNITS (if any)

Francis Scott Key Medical Center, Baltimore Maryland: Yale University School of Medicine, New Haven, Connecticut: Audie L Murphy Memorial Veterans Hospital, San Antonio, Texas: Scott Evans, Normal Vol. intern, SWD, CC

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Clinical Investigations Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS

PROFESSIONAL

OTHER

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects    ☐ (b) Human tissues    ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided)

This project seeks to elucidate the mechanisms by which oral perceptual experience is generated. Since objective measurement of the various aspects of oral experience is fundamental to this effort, the selection and refinement of appropriate psychophysical methods is a primary and continuing project concern. Currently, the routine assessment of taste is carried out using aqueous solutions representing each of the four basic tastes. Measures include both (detection) thresholds and judgments of intensity for taste stimuli at higher, more commonly encountered levels of strength. Assessments of sensitivity to localized taste and touch on the tongue and to variation in the temperature or viscosity of an oral bolus are also available. Olfactory function is routinely assessed by a standardized test of odor identification. These methods are used to study oral sensory changes that may occur with oral or systemic disease and its treatment, with salivary gland dysfunction, with aging or in association with an isolated oral or taste complaint. Such studies lead to an increased understanding of the sensory mechanisms that normally provide for the perception of the complex oral stimuli encountered in everyday life but may, in other circumstances, produce distressing and debilitating oral symptoms.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 DE 00332-13 CI

PERIOD COVERED

October 1, 1993 - September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Oral Medicine Program: Clinical Investigations and Case Reports

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Kohn, William G.	Dep Clinical Director	CIPC	NIDR
Adesanya, Margo	Clinical Associate	CIPC	NIDR
Brahim, Jaime S.	Senior Staff Dentist	CIPC	NIDR
Baum, Bruce J.	Clin. Director/Chief	CIPC	NIDR
Fox, Philip	Chief, CIS	CIPC	NIDR
Grisius, Margaret	Clinical Associate	CIPC	NIDR
Hong, Irene	Clinical Associate	CIPC	NIDR
McCarthy, George M.	Senior Staff Dentist	CIPC	NIDR
Meehan, Sean	Clinical Associate	CIPC	NIDR
O'Connell, Anne	Senior Staff Dentist	CIPC	NIDR
Tessler, Sara	Clinical Associate	CIPC	NIDR
Wu, Ava	Senior Staff Dentist	CIPC	NIDR

COOPERATING UNITS (if any)

Lab. of Clin. Sci., NIMH; Pediatrics Branch, NCI; Inter-Inst. Genet. Prog., CC;  
 Clinical Pathology Lab, CC; Clin. Genet. Sect., NCI.

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Patient Care and Clinical Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS

3.19

PROFESSIONAL

3.09

OTHER

0.10

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects      ☒ (b) Human tissues      ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Clinical case studies of unusual interest and patient-related research are conducted on a variety of oral and dentally-related subjects within the context of our Oral Medicine training program. The CIPCB sponsors an Oral Medicine Fellowship that provides hospital-trained dentists (1-2/year), interested in a career in academic oral medicine and dental research, with a 3-year, high-quality clinical and basic science research experience. Research techniques utilized range from chart and literature reviews, to the direct evaluation and utilization of advanced diagnostic and therapeutic regimens. Interesting problems of oral pathology with or without other medical complications, are often seen in the consult clinic. The study and publication of such cases provide valuable information for the practicing dental clinician as well as a rich training experience for the Oral Medicine fellows. The NIDR clinic is a Dental/Oral Medicine consult clinic for all the NIH institutes. Fellows participate in the diagnosis and treatment of medically-compromised patients referred to the clinic and are encouraged to participate in studies of the oral manifestations of systemic disorders and of the oral complications of medical therapy. Particular emphasis is placed on oral mucosal disorders, with special attention to potential saliva/mucosal interactions. The biological/pathophysiologic implications of clinical conditions are discussed and framed into scientific questions with the help of a staff mentor. Collaborations with investigators at other institutes, utilizing the unique patient populations present at NIH, are encouraged.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER  
Z01 DE 00336-13 CI

PERIOD COVERED

October 1, 1993 - September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Salivary Gland Secretion Mechanisms During Normal and Altered Functional States

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Baum, B.J.	Clinical Dir/Chief	CIPC	NIDR
Adesanya, M.	Clinical Associate	CIPC	NIDR
He, X.	Visiting Associate	CIPC	NIDR
Hiramatsu, Y.	Visiting Associate	CIPC	NIDR
Lalowski, K.	Visiting Associate	CIPC	NIDR
Li, J.	Visiting Fellow	CIPC	NIDR
O'Connell, B.	Staff Fellow	CIPC	NIDR
Park, C.	Visiting Fellow	CIPC	NIDR

COOPERATING UNITS (if any)

CP, CC, NIH; DNM, CC, NIH; Dept. of Nuclear Med., Univ. of Chicago; Dept. of Oral Biology, Boston Univ.; Dept. of Neurology, Cornell Univ.; Dept. of Pulmonary and Critical Care Med., Cornell Univ.; Dept. of Cell Biology, Aarhus Univ., Denmark

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Gene Transfer Unit

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS.

6.73

PROFESSIONAL

5.48

OTHER

1.25

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The health of the oral cavity is maintained by salivary secretions. The principal function of salivary glands is to produce these complex fluids. We utilize a variety of tools to understand saliva formation. During this reporting period, we have completed studies directed at the in vivo identification of muscarinic-cholinergic receptors. These experiments, including a major clinical protocol, have employed stereoisomeric forms of the muscarinic antagonist IQNB. We showed that these agents are useful in vivo tools in brain tissue (mostly  $m_1$ -subtype) but not useful in  $m_3$ -rich tissues like salivary glands. Our focus shifted notably this year with a major emphasis on expanding our efforts on exogenous gene transfer. During this year, we have constructed a recombinant adenovirus containing the cDNA for histatin 3, a potent anticandidal peptide, to use for gene therapeutic purposes. We have also begun construction of an adenovirus containing a cDNA encoding a water channel protein (AQP-CHIP). Since adenovirus-mediated gene transfer is transient in salivary cells, we have initiated efforts to employ adeno-associated virus for gene transfer. We have also continued our studies to understand salivary gland water permeability. Specifically we have shown that the prototypical water channel, AQP-CHIP, is not expressed by salivary epithelial cells and begun studies to identify the molecule(s) involved in transepithelial water movement. We have also shown that the A5 salivary duct cell line inappropriately expresses AQP-CHIP. Finally we have begun a substantial effort to understand polarization and protein routing signals in salivary cells. Of note, we have established the first in vitro model suitable for such studies (SMIE cells, grown on Transwell filters in low glucose medium). The cells show a modest transepithelial electrical resistance, a modest capacity to act as a barrier to [ $^{14}$ C] mannitol flux, and a polarized distribution of the  $\alpha_1$  subunit of the Na/K ATPase.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00337-13 CI

PERIOD COVERED

October 1, 1993 - September 30, 1994

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Oral Physiological Processes: Normal Function and Disease Perturbation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Fox, Philip C.	Dental Officer/Chief, CIS	CIPC	NIDR
Adesanya, Margo	Dental Staff Fellow	CIPC	NIDR
Aladib, Walid	Visiting Fellow	CIPC	NIDR
Ambudkar, Indu	Biologist/Chief, SPS	CIPC	NIDR
Baum, Bruce J.	Clinical Dir/Chief, CIPC	CIPC	NIDR
Kurrasch, Regina H.	Expert	CIPC	NIDR
Macynski, Alice A.	Research Nurse	CIPC	NIDR
(see the attached continuation sheet)			

COOPERATING UNITS (if any)

ARB, NIAMS; DR, CC; DD, NIDDK; DVP, CBER, FDA; Univ. of California, San Francisco, CA; Baylor Univ., Dallas, TX; Howard Univ., Washington, DC; Univ. of Missouri, Kansas City, MO; Univ. of Liverpool, Liverpool, England.

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Clinical Investigations Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS

PROFESSIONAL

OTHER

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects    ☒ (b) Human tissues    ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

This project examines the function of the salivary glands and other oral tissues in individuals with alterations of normal oral function due to disease or therapeutic procedures. Entry into all studies is through the Dry Mouth Clinic. Utilizing outpatient and inpatient services, specific evaluative and diagnostic approaches establish the extent and causes of salivary gland dysfunction in the "dry mouth" patient. The focus of clinical studies is on individuals with primary Sjögren's syndrome, a systemic autoimmune exocrinopathy, with major manifestations of chronic, progressive salivary and lacrimal gland dysfunction. Oral and secretory effects of other selected systemic diseases also are evaluated. An ongoing therapeutic protocol is evaluating the effectiveness of the combination of the anti-inflammatory drug hydroxychloroquine and the parasympathomimetic secretagogue pilocarpine HCl for treatment of salivary, lacrimal, and serological disease in primary Sjögren's syndrome patients. Clinical research studies are i) seeking better salivary and serologic markers of exocrine disease activity in this disorder; ii) defining the expression of adhesion molecules in the salivary glands of patients with different severity of Sjögren's syndrome and normal controls; and iii) evaluating disease progression over time. Laboratory studies focus on the immunopathological mechanisms of salivary dysfunction found in Sjögren's syndrome. The effects of cytokines and other immune mediators on a cultured human salivary ductal cell line have been investigated. This cell line may prove to be a valuable model for study of mechanisms of immune-mediated salivary dysfunction. Work has continued on an in vivo animal model system for inflammatory salivary gland disease using transplantation of tissues into an immunodeficient mouse strain.





Professional Personnel, continued

Sun, Di	IRTA Fellow	CIPC	NIDR
Weiffenbach, James M.	Research Psychologist	CIPC	NIDR
Wu, Ava J.	Senior Staff Dentist	CIPC	NIDR





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00412-09 CI

PERIOD COVERED

October 1, 1993 - September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Oral Endosseous Titanium Implants in Edentulous and Ectodermal Dysplasia Patients.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Brahim, Jaime S.	Senior Staff Dentist	CIPC	NIDR
Folio, John	Consultant	CIPC	NIDR
Kohn, William G.	Chief, PCCSS	CIPC	NIDR
McCarthy, George R.	Senior Staff Dentist	CIPC	NIDR
O'Connell, Brian	Staff Fellow	CIPC	NIDR

COOPERATING UNITS (if any)

Rehabilitation Medicine Department CC; Nutrition Department, CC; Surgical Services Department, CC; Commissioned Officers Dental Clinic, CC; Nursing Department, CC.

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Patient Care and Clinical Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS

3.51

PROFESSIONAL

1.43

OTHER

2.08

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects    ☒ (b) Human tissues    ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project examines the use of endosseous, root form, dental implants in completely edentulous patients, pre-adolescent, adolescent, and adult patients with ectodermal dysplasia (ED), and in adult patients (over 18 years) that require the replacement of single teeth. The implants are utilized to support a fixed dental prosthesis. Removable dentures are considered a significant handicap related to mastication, speech, esthetics, continued reduction of the residual ridges of the mandible and maxillae, and body self-image. Individuals affected by ectodermal dysplasia can have multiple congenitally absent teeth. Consequently, the alveolar bone fails to achieve normal height and volume, as it is dependent upon the development and eruption of the teeth. A lack of alveolar bone not only makes removable denture wear extremely difficult, but also the placement of endosseous implants problematic and potentially less successful. These studies attempt to determine if: (1) endosseous dental implants can be used successfully in non-ED edentulous adult patients and in pre-adolescent, adolescent, and adult ED patients with multiple congenitally missing teeth; and (2) coating a titanium alloy implant with hydroxyapatite improves its success when used to replace single missing teeth. Additionally, we will assess over the duration of this 5-year study if an implant-supported fixed denture significantly affects an individual's loss of vertical dimension of occlusion, satisfaction with treatment, food choice and nutrition, perception of ease/difficulty of chewing selected foods, and body self-image when compared to treatment with a conventional removable denture. Information concerning the relationship of personality to body image and the ability to adapt to oral prostheses of various types will also be assessed. Finally, the project will evaluate the effects, if any, that mandibular endosseous dental implants have on the growth and development of the craniofacial complex of pre-adolescent (7-11 year-old) patients with ED and significant hypodontia.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 DE 00415-09 CI																												
PERIOD COVERED October 1, 1993 - September 30, 1994																														
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders ) Ion Transport and Fluid Secretion in Salivary Glands																														
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator ) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 30%;">Turner, R. J.</td> <td style="width: 30%;">Chief/MTS</td> <td style="width: 20%;">CIPC</td> <td style="width: 20%;">NIDR</td> </tr> <tr> <td>Moore, M.</td> <td>Staff Fellow</td> <td>CIPC</td> <td>NIDR</td> </tr> <tr> <td>Evans, R.</td> <td>Visiting Fellow</td> <td>CIPC</td> <td>NIDR</td> </tr> <tr> <td>Tanimura, A.</td> <td>Visiting Fellow</td> <td>CIPC</td> <td>NIDR</td> </tr> <tr> <td>Ferri, C.</td> <td>Special Volunteer</td> <td>CIPC</td> <td>NIDR</td> </tr> <tr> <td>Brookes, N.</td> <td>Guest Worker</td> <td>CIPC</td> <td>NIDR</td> </tr> <tr> <td>Tessler, S.</td> <td>Clinical Associate</td> <td>CIPC</td> <td>NIDR</td> </tr> </table>			Turner, R. J.	Chief/MTS	CIPC	NIDR	Moore, M.	Staff Fellow	CIPC	NIDR	Evans, R.	Visiting Fellow	CIPC	NIDR	Tanimura, A.	Visiting Fellow	CIPC	NIDR	Ferri, C.	Special Volunteer	CIPC	NIDR	Brookes, N.	Guest Worker	CIPC	NIDR	Tessler, S.	Clinical Associate	CIPC	NIDR
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Tanimura, A.	Visiting Fellow	CIPC	NIDR																											
Ferri, C.	Special Volunteer	CIPC	NIDR																											
Brookes, N.	Guest Worker	CIPC	NIDR																											
Tessler, S.	Clinical Associate	CIPC	NIDR																											
COOPERATING UNITS (if any)																														
LAB/BRANCH Clinical Investigations and Patient Care Branch																														
SECTION Membrane Biology Section																														
INSTITUTE AND LOCATION NIDR, NIH, Bethesda, MD 20892																														
TOTAL STAFF YEARS 4.13	PROFESSIONAL 4.13	OTHER 0.00																												
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects              <input type="checkbox"/> (a1) Minors              <input type="checkbox"/> (a2) Interviews         </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues         </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither         </div> </div>																														
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) <p>Saliva is the principal protective agent for the mouth and thus is of primary importance to oral health maintenance. Perturbations of salivary secretory mechanisms can consequently lead to serious oral health problems. The objective of this project is to study the membrane and cellular processes which underlie the phenomenon of salivary fluid secretion and thus to contribute to our understanding of the fluid secretory process in normal and diseased states. Because similar secretory mechanisms are thought to be common to a number of other exocrine glands, this information should be of rather broad applicability and interest. During the present reporting period our specific areas of focus were the following: (1) Studies of the regulation of the rat parotid acinar Na-K-2Cl cotransporter by secretagogues and other stimuli were continued at the levels of transport activity and protein phosphorylation. (2) Production of monoclonal antibodies against extramembrane sites on the parotid Na-K-2Cl cotransporter were begun. (3) The (putative) rat parotid Na-K-2Cl cotransporter was partially cloned and sequenced. (4) The effects of free radicals on cell spreading were investigated in the human salivary ductal cell line HSY.</p>																														





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00438-08 CI

PERIOD COVERED

October 1, 1993 - September 30, 1994

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Molecular Mechanisms Regulating  $\text{Ca}^{2+}$  Flux in Salivary Glands

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Ambudkar, I.	Chief/SPS	CIPC	NIDR
Baum, J.B.	Clin Dir/Chief	CIPC	NIDR
Hiramatsu, Y.	Visiting Associate	CIPC	NIDR
Lockwich, T.	Senior Staff Fellow	CIPC	NIDR
Sawaki, K.	Visiting Associate	CIPC	NIDR
Hong, I.	Clinical Associate	CIPC	NIDR
Meehan, S.	Clinical Associate	CIPC	NIDR

(see attached continuation sheet)

COOPERATING UNITS (if any)

Department of Biological Chemistry, University of Maryland School of Medicine;  
Division of Nephrology; Dept. Medicine, Johns Hopkins Univ. School of Medicine;  
LCB, NIDDK; LCMB, NIA.

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Secretory Physiology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS

5.44

PROFESSIONAL

5.31

OTHER

0.13

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is directed towards understanding the processes which regulate cytosolic  $[\text{Ca}^{2+}]$  in salivary gland cells. Previously we have described a phosphatidylinositol-4,5,bisphosphate specific phospholipase C enzyme (PLC) in rat parotid gland membranes, which is independently regulated by both  $m_3$ -muscarinic and  $\alpha_1$ -adrenergic receptors via  $\alpha$  subunits of the  $G_{q/11}$  family of G-proteins. In this reporting period we have demonstrated that receptor-dependent stimulation of this PLC is decreased in old rat parotid gland membranes, compared to young rat membranes, which can be accounted for by a defect in activation of  $G\alpha_{q/11}$ . We have also shown earlier that the  $\text{Ca}^{2+}$  entry mechanism in rat parotid acinar cells, which is critical for prolonged fluid secretion in this gland, belongs to the " $\text{Ca}^{2+}$  release-activated  $\text{Ca}^{2+}$  entry" type of  $\text{Ca}^{2+}$  influx found in a number of non-excitabile cells. This largely uncharacterized mechanism appears to be stimulated by depletion of  $\text{Ca}^{2+}$  in the intracellular  $\text{Ca}^{2+}$  store(s). In this reporting period we have demonstrated that this  $\text{Ca}^{2+}$  influx is regulated by phosphorylation (inhibition) and dephosphorylation (stimulation). We have also studied the kinetics of divalent cation ( $\text{Ca}^{2+}$  and Mn) influx into parotid acini. Unstimulated acini have apparent low ( $K_d=750\mu\text{M}$ ) and high ( $K_d=6\mu\text{M}$ ) affinity sites for Mn and a single site ( $K_d=3.8\text{mM}$ ) for  $\text{Ca}^{2+}$ . In internal  $\text{Ca}^{2+}$  pool-depleted acini the  $V_{\text{max}}$  of these sites is increased and, in addition, there is a high affinity site for  $\text{Ca}^{2+}$  ( $K_d=150\mu\text{M}$ ). The high affinity Mn influx site is inhibited by miconazole. Importantly, we have detected a high affinity ( $K_d=200\mu\text{M}$ ), temperature and miconazole-sensitive,  $\text{Ca}^{2+}$  flux component in isolated basolateral membrane vesicles. We have solubilized this component with octylglucoside, and reconstituted it into liposomes made of *E.coli* bulk phospholipids.  $\text{Ca}^{2+}$  flux in the reconstituted proteoliposomes exhibits similar characteristics, e.g., divalent cation & DCCD sensitivity, as the native vesicles.





## Professional Personnel, continued

Sakai, T.	Visiting Fellow	CIPC	NIDR
Chauthaiwale, J.	Visiting Fellow	CIPC	NIDR
Taylor, S.	Special Volunteer	CIPC	NIDR
O'Connell, Anne	Senior Staff Dentist	CIPC	NIDR



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00049-23 LCDO

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Physiological Function of Transglutaminases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Chung, Soo Il	Research Chemist	LCDO, NIDR
Others:	Kang, Kee Ryeon	Visiting Fellow	LCDO, NIDR
	Hwang, Hi Ku	Guest Researcher	LCDO, NIDR
	Folk, John E.	Chief, ECS	LCDO, NIDR

COOPERATING UNITS (if any)

Peter Steinert, Laboratory of Skin Biology, NIAMS  
Soo Yeol Kim, Laboratory of Skin Biology, NIAMS  
Franco Carmassi, 2nd Medical Clinic, Pisa University, Pisa, Italy

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Enzyme Chemistry Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.25

PROFESSIONAL:

2.25

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minor  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The N<sup>ε</sup>(γ-glutamyl)lysine crosslinking reaction which is catalyzed by the transglutaminases (TGases) provides structural integrity to the epithelium, activates latent TGF-β and inactivates IL-2. TGase K, epithelial TGase, is involved in terminal differentiation in stratified squamous epithelium through formation of the cornified cell envelope. In an effort to understand the control and regulation of the differentiation process in epithelium, we have expressed this enzyme and a series of deletion forms of the enzyme and have related enzymatic properties to molecular structure. Inactive native proenzyme is presumably normally activated by proteases. A deletion form with residues 1-62 removed displayed partial activation, whereas one with residues 1-97 removed was 10-fold more active than the 1-62 deletion form toward certain protein substrates, but far less active toward others and toward small peptides. This is consistent with the knowledge that TGase K is activated in human keratinocytes by cleavages at Arg-Gly bonds between residues 92-93 and 572-573 to produce the 58 kDa active enzyme. Together these findings indicate that structural features between residues 63-92 have a strong influence on substrate specificity. It is now possible to design and produce an enzyme with defined substrate specificity.

On occasion, tuberculosis patients being treated with isoniazid develop severe hemorrhagic symptoms. The IgG fraction from such a patient was found to inhibit fibrin crosslinking and to react in an auto-immune fashion with the C-terminal crosslinking sites of the fibrin γ-chains. These observations provided a potential for obtaining an epitope effective in controlling thrombosis in certain vascular disease.





DEPARTMENT OF HEALTH AND HUMAN SERVICES • PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00311-14 LCDO

PERIOD COVERED

October 1, 1993 - September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Essential Cellular Function of Hypusine in eIF-5A\*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Park, Myung Hee Research Chemist LCDO, NIDR

Others: Joe, Young Ae Visiting Fellow LCDO, NIDR

COOPERATING UNITS (if any)

Dr. Philip Coffino, University of California, San Francisco, CA.

Dr. John W.B. Hershey, University of California, Davis, CA

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Enzyme Chemistry Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.34

PROFESSIONAL:

1.34

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Eukaryotic protein translation initiation factor 5A (eIF-5A) contains one residue of hypusine and appears to be the only cellular protein with this unique amino acid. Hypusine is produced post-translationally by transfer of the butylamine portion of the polyamine spermidine to a lysyl residue in the eIF-5A precursor to form deoxyhypusine followed by hydroxylation to form hypusine.

The precise physiological role of the hypusine-containing protein eIF-5A is yet unknown. However, it is well established that hypusine is vital for eukaryotic cell proliferation. The marked enhancement of hypusine formation upon mitogen treatment of cells, the requirement for the hypusine modification in eIF-5A for yeast viability and the inhibition of cell proliferation by inhibitors of hypusine biosynthesis support this notion. The strict conservation of the sequence of twelve amino acids surrounding the hypusine residue further emphasizes the importance of this residue. The basis for the specificity of hypusine synthesis with respect to the substrate protein was investigated using fragments of eIF-5A precursor protein as substrates for deoxyhypusine synthase, the first enzyme in hypusine biosynthesis. These were generated either by specific endoproteinases or by recombinant deletion subcloning. The results define the minimum domain of the eIF-5A precursor protein required for deoxyhypusine synthesis as Phe<sup>30</sup>-Asp<sup>80</sup> and provide insight into the molecular interaction between eIF-5A and deoxyhypusine synthase.

In an effort to identify other cellular proteins with which eIF-5A interacts to exert its biological activity, two-hybrid system based cloning was initiated. In addition, the involvement of eIF-5A and hypusine in the activity of rev, the regulatory protein required for HIV-1 replication is under investigation.

\* Previous title: Hypusine in eIF-4D: Biosynthesis and Function





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00433-08 LCDO

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Functional Aspects of C-reactive Protein

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Robey, Frank A. Chief, PIU LCDO, NIDR

Others: Ivanov, Boris Visiting Fellow LCDO, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Peptide and Immunochemistry Unit

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.25

PROFESSIONAL:

1.25

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

C-reactive protein (CRP) and serum amyloid P component (SAP) are two closely related proteins with respect to their primary structure and their pentameric appearance under the electron microscope. The two proteins have known functions and a common property shared by CRP and SAP is their ability to bind sulfated polysaccharides and to fibronectin in a calcium-dependent manner. In addition, both proteins have peptide subunits that may possess immunomodulating activities.

We are continuing our efforts on developing synthetic subunits of CRP and SAP for use as possible immunomodulators and cell surface recognition materials. For these studies, new peptide chemistry to make conformationally constrained derivatives are being developed.

BBPA is a new heterotrifunctional cross-linking reagent that allows for the specific cross-linking of a peptide by placing a bromoacetyl moiety at any position in a peptide chain. BBPA is a second generation of bromoacetyl-derivatized materials that provide limited spacing between the peptide of interest and its conjugate partner. In contrast to the 8-carbon spacing provided by BBAL which we synthesized earlier, BBPA allows for only a 2 carbon spacing. We have developed BBPA into a white powder that can be easily weighed out and handled. Several model RGD-containing peptides and conjugates have been synthesized containing BBPA. A cyclic peptide made with BBPA containing the amino acid sequence of human bone sialo protein was found to possess an activity for supporting osteoblast adhesion that was comparable to the native protein.

Such new compounds form the foundations for the design and syntheses of countless new materials that broaden our repertoire of research tools in the lab and contribute to technologies that are used in the private sector.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01DE00434-08 LCDO
PERIOD COVERED October 1, 1993 to September 30, 1994		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies on HIV-1 Targeted Drug Delivery System		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Robey, F.A.	Chief, PIU LCDO, NIDR
Others:	Harris-Kelson, Tracy Ivanov, Boris Liu, Mingfang	Staff Fellow Visiting Fellow Visiting Fellow LCDO, NIDR LCDO, NIDR LCDO, NIDR
COOPERATING UNITS (if any) Majorie Robert-Guroff, National Cancer Institute; Peter Roller, NCI; Marian Neutra, Harvard University; Thomas VanCott; WRAIR		
LAB/BRANCH Laboratory of Cellular Development and Oncology		
SECTION Peptide and Immunochemistry Unit		
INSTITUTE AND LOCATION NIDR, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 2.75	PROFESSIONAL: 2.75	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.) <p>HIV-1 is the causative agent of AIDS. CD4 is the cellular receptor for HIV-1 and its amino acid sequence is known. The regions of HIV-1 that binds to CD-4 is termed gp160 and this is the envelope glycoprotein that is composed of gp120 and gp41. The gp120 region specifically binds to CD4 and the sequences of amino acids in both CD4 and gp120 that are responsible for the high affinity binding of the virus are now known. Peptomer (419-436) has been tested for immunogenicity in rabbits and in rhesus monkeys. The rabbits were found to produce antibodies that cross reacted with recombinant gp120 when the helical conformation of the peptomer was conserved; antibodies against the nonhelical conformation did not react with gp120. The monomer peptide was not immunogenic in the rabbit.</p> <p>The rhesus monkeys we used were pre-immunized with various cocktails of gp120 and gp160 in various formulae and in an adeno virus expression system. These animals displayed protective antibodies against HIV-1 but the levels of these antibodies had diminished to non-protective levels within a few months. The monkeys never had antibodies that recognized peptomer (419-436). When we immunized the monkeys with peptomer (419-436) in either alum or in phosphate-buffered saline, a new immune response was produced and the <u>in vitro</u> neutralizing response was restored.</p> <p>We tested HIV+ human serum for antibodies to peptomer (419-436) and learned that, due to hypergammaglobulinemia there was a very high background that was found to be nonspecific. In addition, 20 normal humans who were HIV- but who were immunized with Genetech's gp120 did not produce antibodies that recognized peptomer (419-436). The results indicate that peptomer (419-436) can provide a unique set of antibodies against an epitope on HIV-1 that neither natural infection nor the current envelope vaccines elicit immune responses against.</p>		





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1DEOO479-06 LCDO

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Mechanisms Responsible for Oncogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Robbins, Keith C.	Chief, MCBS	LCDO, NIDR
Others:	Matoskova, Brana	Visiting Fellow	LCDO, NIDR
	Marcilla-Diaz, Antonio	Visiting Fellow	LCDO, NIDR
	Jakus, Judit	Visiting Fellow	LCDO, NIDR
	Geiser, Jeanne	Chemist	LCDO, NIDR
	Edward, Stephens	Biolab Tech	LCDO, NIDR

COOPERATING UNITS (if any)

Paolo DiFiore, LCMB, NCI; Oliver A. Sartor, LSU Health Center at Schreveport, LA

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Molecular and Cellular Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

3.71

PROFESSIONAL:

3.05

OTHER:

.66

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The section continues to investigate mechanisms underlying the conversion of normal cells to malignant status. Novel mechanisms and the molecules they involve become candidates for evaluation in our biologic models which include murine fibroblasts, nude mice, human squamous carcinoma cell lines and human tumor tissue. During the current reporting period, we have established a model system for identification of tyrosine phosphorylated molecules required for the malignant transformation of murine NIH/3T3 cells. Efforts to isolate these molecules are in progress.

The EGF receptor is constitutively active in a high fraction of head and neck squamous cell carcinomas. Thus, substrates for the enzyme are also likely to be in active form in neoplastic cells. In collaboration with Dr. Pier Paolo DiFiore of the National Cancer Institute, we are investigating the nature of EGF receptor substrates and the biologic effect they impart when switched on. To date a number of substrates have been identified by virtue of their tyrosine phosphorylation upon EGF treatment of cells expressing the receptor.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZOIDE00480-06 LCDO

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Normal Physiologic Roles for Nonreceptor Protein-Tyrosine Kinases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Robbins, Keith C.	Chief, MCBS	LCDO, NIDR
Others:	Rivero, Octavio	Visiting Fellow	LCDO, NIDR
	Teramoto, Hidemi	Visiting Fellow	LCDO, NIDR
	Geiser, Jeanne	Chemist	LCDO, NIDR
	Stephens, Edward	Bio Lab Tech	LCDO, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Molecular and Cellular Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.80

PROFESSIONAL:

2.14

OTHER:

.66

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Section's long standing interest in non-receptor protein-tyrosine kinases has re-focused within the last few years on myelomonocytic cells, major players in periodontal disease. Our recent work has demonstrated that protein-tyrosine phosphorylation functionally links activation of Fc receptors and degranulation. Furthermore, a novel protein-tyrosine kinase, designated syk, has been shown to be a major player in the process signalling occupation of Fc receptors by antigen-antibody complexes. Another aspect of this project involves identification of effectors and substrates for protein-tyrosine kinases. Our approach has involved the use of protein-protein interactions to isolate and characterize molecules in the signalling pathways utilizing non-receptor protein-tyrosine kinases. During the current reporting period, we have characterized interactions mediated by regulatory domains of src-family kinases and have taken advantage of this information to clone putative effectors and substrates from cDNA expression libraries prepared from macrophages. Two molecules have been isolated, one of which has no homologue in the protein data bases. The other was surprisingly the protein product of the cbl proto-oncogene. The relationship between cbl and functional signalling pathways in myelomonocytic cells is in progress.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00551-03 LCDO

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of G Proteins in Growth Control and Carcinogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Gutkind, J Silvio	Chief, MSU	LCDO, NIDR
Others:	Robbins, Keith C.	Chief, LCDO	LCDO, NIDR
	Xu, Ningzhi	Visiting Associate	LCDO, NIDR
	Coso, Omar	Visiting Fellow	LCDO, NIDR
	Crespo, Piero	Guest Researcher	LCDO, NIDR
	Gellineau, Victor	Biologist	LCDO, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Molecular Signalling Unit

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

4.89

PROFESSIONAL:

4.05

OTHER:

.84

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our interest is in the molecular basis of cancer. We have approached this problem by studying biochemical pathways that participate in the transduction of proliferative signals. Current focus is in the role of G proteins and their coupled receptors in normal cell growth and oncogenes. In previous studies, we have shown that mutated genes for certain classes of G proteins are as transforming as the most potent known oncogenes. Consistent with this observation was our finding that cell surface receptors functionally coupled to these G proteins can induce malignant transformation in an agonist dependent manner. We then began dissecting biochemical pathways utilized by transforming G protein-coupled receptors. We found that one such pathway involves the exchange of GDP for GTP in the protein product of the *ras* proto-oncogene. The nucleotide exchange in p21<sup>ras</sup> leads to the activation of the p72<sup>raf</sup> kinase, and both events were found to be induced in a PKC-independent manner. Furthermore, activation of *ras* and *raf* was found to be strictly required to induce DNA synthesis or transformation by G protein-coupled receptors. Upon activation, p72<sup>raf</sup> is believed to be one of the initiators of a cascade of serine-threonine kinases that converges in the activation of MAP kinases, which ultimately regulate the expression of genes essential for proliferation. In line with our previous results, we found that G protein-coupled receptors potently activate MAP kinases in a PKC-independent fashion.

We next investigated how G proteins link cell surface receptors to the activation of the MAP kinase cascade. A tenant of the field has held that  $\alpha$  subunits are the active components of G proteins and that the  $\beta\gamma$  complex simply regulates  $\alpha$  activity. However, we have found that  $\beta\gamma$  subunits of G proteins, not  $G\alpha$ , act in a *ras*-dependent manner to stimulate MAP kinases. These findings are likely to constitute a milestone in the study of signal transduction, and support a novel mechanism for activation of *ras* by  $\beta\gamma$  complexes. Furthermore, our observations suggest that signals from G protein-coupled receptors converge at the level of p21<sup>ras</sup> with the pathway utilized by receptor-tyrosine kinases.

We have previously shown that the  $\alpha$  subunit of the novel G<sub>12</sub> protein can behave as a potent dominant acting oncogene. In an effort to determine whether this G protein plays a role in human neoplasia we have cloned human genes for both members of the G<sub>12</sub> family, G<sub>12</sub> and G<sub>13</sub>. We found that overexpression of the human cDNA for either G protein  $\alpha$  subunit is sufficient to cause malignant transformation. Furthermore, a systematic screen of tumor-derived cell lines suggests that alterations in the expression of these G proteins might contribute to human neoplasia, particularly in adenocarcinoma of the breast. Animal models are being currently developed to explore whether these genes can alter normal functioning or are tumorigenic when expression is targeted to the mammary or salivary glands.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01DE00558-03 LCDO
PERIOD COVERED October 1, 1993 to September 30, 1994		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Oral Carcinogenesis		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Robbins, Keith C.	Chief, MCBS LCDO, NIDR
OTHERS:	Cardinali, Massimo	Visiting Associate LCDO, NIDR
	Yeudall, William A.	Visiting Associate LCDO, NIDR
	Geiser, Jeanne	Chemist LCDO, NIDR
	Stephens, Edward	Bio Lab Tech LCDO, NIDR
	Winn, Debbie	Chief, ASHAB EODPP, NIDR
	Schwartz, Joel	Senior Dental Scientist EODPP, NIDR
COOPERATING UNITS (if any) John Ensley, Wayne State University		
LAB/BRANCH Laboratory of Cellular Development and Oncology		
SECTION Molecular and Cellular Biology Section		
INSTITUTE AND LOCATION NIDR, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 2.73	PROFESSIONAL: 2.05	OTHER: .68
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>             The LCDO's Oral Cancer Program investigates etiology, diagnosis, treatment and prevention of oral tumors, placing a special the emphasis on the most prevalent oral cancer, squamous cell carcinoma (SCC). One component of this effort involves identification of molecules that uniquely describe stages of progression from normal to malignant. The presence or absence of such molecules can be useful information for (i) diagnosis, (ii) in determining appropriate treatment modalities and (iii) in assessing the efficacy of chemoprevention protocols. Among the growth promoting molecules identified to date as markers of malignancy are the receptor for epidermal growth factor in combination with one or more of its agonists. Of the growth regulatory class of molecules, p53 is nonfunctional in &gt;90%of SCCs, and an inhibitor of cyclin kinase, p16, is absent in a similar fraction of tumors. Thus, the laboratory's repertoire of molecular markers heralding the malignant state continues to expand. Assays developed for such molecules will be useful to others clinically and to us as intermediate biomarkers for assessing progress in chemoprevention settings.           </p>		





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01DE000605-01 LCDO
PERIOD COVERED October 1, 1993 - September 30, 1994		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Inhibitors of Hypusine Biosynthesis		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Folk, John E.	Chief, ECS LCDO, NIDR
Others:	Park, Myung-Hee	Research Chemist LCDO, NIDR
	Wolff, Edith C.	Expert LCDO, NIDR
	Lee, Young-Bok	Visiting Fellow LCDO, NIDR
	Jakus, Judit	Visiting Fellow LCDO, NIDR
COOPERATING UNITS (if any) Dr. H. Hanuske-Abel and others, Cornell University, Medical College- The New York Hospital, New York, NY.		
LAB/BRANCH Laboratory of Cellular Development and Oncology		
SECTION Enzyme Chemistry Section		
INSTITUTE AND LOCATION NIDR, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.92	1.92	
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) <p>The unique amino acid hypusine occurs only in one cellular protein, eukaryotic protein translation initiation factor 5A (eIF-5A), and only at a single site. Because hypusine is formed posttranslationally and since both eIF-5A and its hypusine modification appear to be vital for cell growth, we have aimed our attention toward inhibition of hypusine formation as a potential means of controlling eukaryotic cellular proliferation. The two enzymatic steps in hypusine production, deoxyhypusine synthesis and deoxyhypusine hydroxylation offer prime targets for intervention and several inhibitors for the enzymes that catalyze these steps were formulated and tested for their cellular effects.</p> <p>Guanyl diamines modeled after quazatine, a quanylated polyamine with broad spectrum activity against seed-borne fungi and citrus mold which displayed some inhibition against the enzyme deoxyhypusine synthase, proved to be potent inhibitors of both this enzyme and cellular proliferation and provided the basis for a recent U.S. patent entitled "Compositions and methods for inhibiting deoxyhypusine synthase and the growth cells". Evidence that these inhibitors exert their action by competing with spermidine for binding to the enzyme and that they act in an antiproliferative manner through intercellular inhibition of hypusine biosynthesis places them in a novel class of antiproliferative agents. Work is underway to design and test new members of this class of agents. Among these are oxyamines, quanidinated oxy- and thioethers, amidines, and isothiureas.</p> <p>Certain metal chelators, notably L-mimosine, prevent hypusine formation through inhibition of deoxyhypusine hydroxylase. Suppression of hypusine formation by inhibition of this enzyme correlates with arrest of cell proliferation resulting in accumulation of cells in the late G1 phase of the cell cycle. Since L-mimosine also causes strong inhibition of prolyl 4-hydroxylase, arrest of proline-to-hydroxyproline conversion and consequently suppression of collagen secretion, it was tested for combined antiproliferative/fibrosuppressive effect on smooth muscle cells of human atherosclerotic coronary arteries. The positive combined effects have important implications for control of fibrosis in general and, in particular, for understanding the pathophysiology of restenosis, the recurrent closing of vessels of the heart following surgical reconstruction.</p>		



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01DE00608-01 LCDO
PERIOD COVERED October 1, 1993 to September 30, 1994		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders ) <b>Deoxyhypusine Synthase: Purification, Characterization and cDNA Cloning</b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Park, Myung Hee	Research Chemist LCDO, NIDR
Others:	Wolff, Edith C.	Expert LCDO, NIDR
	Lee, Young Bok	Visiting Fellow LCDO, NIDR
	Chung, Soo Il	Research Chemist LCDO, NIDR
	Kang, Kee Ryeon	Visiting Fellow LCDO, NIDR
	Folk, John E.	Chief, ECS LCDO, NIDR
COOPERATING UNITS (if any)		
LAB/BRANCH		
Laboratory of Cellular Development and Oncology		
SECTION		
Enzyme Chemistry Section		
INSTITUTE AND LOCATION		
NIDR, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.08	2.08	
CHECK APPROPRIATE BOX(ES)		
<input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)		
<p>An unusual amino acid, hypusine, which occurs on only one cellular protein, eukaryotic translation isolation factor 5A (eIF-5A), is intimately involved in cell proliferation. Hypusine biosynthesis occurs by way of two sequential post-translational modification reactions: i) deoxyhypusine synthesis by deoxyhypusine synthase and ii) deoxyhypusine hydroxylation by deoxyhypusine hydroxylase.</p> <p>The first enzyme, deoxyhypusine synthase, was purified to homogeneity after ~100,000-fold enrichment from rat testis by cell fractionation, ammonium sulfate fractionation, ion exchange chromatography and chromatofocusing. The purified enzyme displays remarkably narrow specificity toward its substrates, spermidine, NAD, and the eIF-5A precursor protein. As shown previously with less pure enzyme, in addition to deoxyhypusine synthesis, the enzyme can carry out a partial reaction, the NAD-dependent cleavage of spermidine, in the absence of the precursor protein. The active enzyme appears to exist as a dimer of 42 kDa subunits, with a pI of 4.75. Partial amino acid sequences were determined from several tryptic peptides isolated from the 42-kDa monomer. Rabbit polyclonal antibodies raised against the 42-kDa protein inhibit deoxyhypusine synthase from rat testis and also cross-react with the human enzyme from HeLa cells. Attempts to clone the cDNA for deoxyhypusine synthase are underway, with the use of the rabbit antiserum and oligonucleotide primers corresponding to the partial amino acid sequences.</p>		





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 DE 00230-18 LDB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Proteins in Tissue Architecture and Cell Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Kleinman, Hynda K.	Section Chief	LDB, NIDR
Others:	Kibbey, Maura C.	Staff Fellow	LDB, NIDR
	Grant, Derrick S.	Visiting Associate	LDB, NIDR
	Schnaper, Howard W.	Special Expert	LDB, NIDR
	Hoffman, Matthew	Visiting Fellow	LDB, NIDR

COOPERATING UNITS (if any)

NIA, NIH, Jucker M; NIAID, NIH, Fauci A; Johns Hopkins Med Sch, Balt MD, Walker L; Georgetown U Med Sch, Wash DC, Dym M; Yale U Med Sch, Rosen E; NIDDK, NIH, Vargas F; Harvard Univ, Boston MA, Neve R; U California, Irvine CA, Van Nostrand W.

LAB/BRANCH

Laboratory of Developmental Biology

SECTION

Cell Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

4.0

PROFESSIONAL:

3.67

OTHER:

0.33

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The extracellular matrix has been found to be important in embryogenesis and in tissue repair. From in vitro studies using purified components, a better understanding of how cells adhere, migrate, proliferate, and differentiate in response to tissue and cell-specific matrix molecules has been established. We have found that the basement membrane, the extracellular matrix which underlies all epithelial cells and endothelial cells and surrounds nerve cells, promotes cell differentiation in vitro. When cultured on basement membrane, endothelial cells form capillary-like structures with a lumen, bone cells form canaliculi, salivary cells form glands, etc. Our goal is to define the molecular and cellular events involved in this process. Our approach has been (1) to identify the biologically active matrix components, (2) localize active sites on the matrix component with site specific antibodies and synthetic peptides, (3) identify and characterize cellular receptors, (4) gain an understanding of the intracellular events involved in the biological response, and (5) identify genes induced by the extracellular matrix. Estrogens have been found to promote leukocyte adhesion to endothelial cell monolayers via an increase in endothelial cell selectin adhesion receptors. This finding may explain the increase in inflammatory diseases in women. In addition, estrogens promote endothelial cell adhesion, growth migration and tube formation in vitro and angiogenesis in vivo. Using the endothelial cell tube assay, a new role for proteases and collagen has been defined and may have important clinical uses in vessel repair. Subtractive cDNA cloning of endothelial cells on plastic vs basement membrane has identified several novel genes as well as thymosin B4 and calmodulin as induced during differentiation into vessels. The laminin-derived peptide SIKVAV promotes neurite outgrowth. A brain derived cellular receptor for SIKVAV shares homology with the amyloid precursor protein and may define the role of this protein in development and in Alzheimer's disease.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00482-06 LDB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Tumor Growth and Metastases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Kleinman, Hynda K.	Section Chief	LDB, NIDR
Others:	Kibbey, Maura C.	Staff Fellow	LDB, NIDR
	Kim, Woo H.	Guest Researcher	LDB, NIDR
	Lentini, Alex	Guest Researcher	LDB, NIDR
	Roque, Eva M.	Biol Lab Tech	LDB, NIDR

COOPERATING UNITS (if any)

UCSF, San Francisco CA, Kim Y; NIA, NIH, Passaniti A; Lombardi Cancer Center, Wash DC, Thompson E; Catholic Univ, Wash DC, Tozeren A.

LAB/BRANCH

Laboratory of Developmental Biology

SECTION

Cell Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.09

PROFESSIONAL:

1.92

OTHER:

0.17

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies are conducted to define the mechanisms involved in tumor growth and metastasis and to develop new animal models of human cancers. We have found that a basement membrane extract (Matrigel) when premixed with human tumor cells (which do not grow well in mice) promotes their incidence and growth. Very low cell numbers can be used. We have been able to culture new highly differentiated human tumor cell lines from the tumors grown in mice including certain colon cell lines and a Nelson's pituitary tumor cell line. Laminin, a major basement membrane component, has been found to promote the malignant phenotype. Selection for adhesion to laminin was carried out with a human colon cancer cell line developed from a patient biopsy passaged in mice with Matrigel and the adherent cells were found to be highly malignant when injected with Matrigel. The laminin non-adherent cells formed few tumors which were highly differentiated.

Various biologically active laminin-derived synthetic peptides have been identified. Previously, we found that YIGSR (tyr-ile-gly-ser-ag) reduced tumor growth, metastases and angiogenesis. We now find multimeric forms of this peptide are more active than the monomers and are able to induce apoptosis. Another laminin-derived peptide containing SIKVAV from the A chain has been found to increase tumor growth, lung colonization, and angiogenesis as well as collagenase IV activity and plasminogen activation. This peptide was found to promote angiogenesis in an in vivo model by increasing the recruitment of neutrophils. Using this information and the newly developed models of human tumors, the development of new therapeutic strategies for cancer should be facilitated.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 DE 00483-06 LDB
PERIOD COVERED October 1, 1993 to September 30, 1994		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Gene Regulation and Function of Cartilage		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Yamada, Yoshihiko Others: Yamada, Kenneth Krebsbach, Paul Nakata, Ken Gao, Luo-yi Lee, Suk Keun Bernier, Suzanne Watanabe, Hideto	Section Chief Laboratory Chief Staff Fellow Visiting Fellow Visiting Associate Visiting Fellow Visiting Fellow Visiting Fellow	LDB, NIDR LDB, NIDR LDB, NIDR LDB, NIDR LDB, NIDR LDB, NIDR LDB, NIDR
COOPERATING UNITS (if any) Shriner's Hospital, Portland, OR; Johns Hopkins University, Baltimore, MD; Aichi Medical University, Osaka, Japan; Wistar Institute, Philadelphia, PA; University of Tennessee, Memphis, TN; NIDDK, NIH.		
LAB/BRANCH Laboratory of Developmental Biology		
SECTION Molecular Biology Section		
INSTITUTE AND LOCATION NIDR, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 4.42	PROFESSIONAL: 3.42	OTHER: 1.00
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>             The aim of this project is to understand the mechanisms underlying cartilage and craniofacial development. We have studied a genetic disease of skeletal and craniofacial abnormalities and identified genes involved in the normal development of cartilage and craniofacial tissues. The major components of cartilage are aggrecan, link protein and type II collagen. Aggrecan interacts with hyaluronic acid and link protein to form aggregates. cDNA clones have been used to isolate and characterize the rat aggrecan gene and its promoter. The gene is about 80 kb in size containing 18 exons, most of which encodes structural modules, exceptions are domains G1-b and G2-B, which are split into two exons; and the G3 lectin domain, which is encoded as three exons. Exon 1 has been mapped and contains 381 bp of CCAAT elements, but has several putative binding sites for SP-1, AP-2 and NFκ-b site. A 920 bp promoter sequence containing 280 bp exon 1 sequence has shown to be active in transfected chondrocytes. Mouse cartilage matrix deficiency (cmd) is an autosomal recessive mutation characterized by cleft palate, short limbs, tail and snout and homozygous mice die just after birth. A 7 bp deletion in exon 5 of the aggrecan gene has been identified in cmd mice. The mutation causes a frame shift that potentially generates a severely truncated molecule that is missing a region essential for binding other components that make up cartilage network. The enhancer sequence of the collagen II gene has been delineated by deletion and site-specific mutation analysis. One of the clones encodes the C-pro-peptide of procollagen II. A potential regulatory role of the C-propeptide for collagen II gene transcription has been studied. A TA rich sequence around -920 of the link protein gene has been identified as one of the elements required for tissue specific transcription of the link protein gene. A glucocorticoid response element has been located in the first intron of the link protein gene. This similar AT rich segment was found in the collagen II enhancer, suggesting that a common nuclear factor in chondrocytes may regulate both collagen II and link protein genes. We have initiated a genome project to identify novel genes involved in craniofacial and tooth development. Two cDNA libraries were constructed using mRNA from mouse embryo maxillofacial tissues and rat incisor. Over 1000 cDNA clones from both libraries have been sequenced. Studies characterizing these clones by examining their stage and tissue-specific expression have been initiated.           </p>		



Project Number Z01 DE 00483-06 LDB

Professional Personnel (continued)

Kimata, Koji  
Rhodes, Craig

Fogarty Scholar  
Biologist

LDB/NIDR  
LDB/NIDR





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 DE 00484-06 LDB
PERIOD COVERED October 1, 1993 to September 30, 1994		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Animal Models of Connective Tissue Disease in Transgenic Mice		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Yamada, Yoshihiko Others: Watanabe, Hideto Nakata, Kenneth Takami, Hiro Krebsbach, Paul Strong, David	Section Chief Visiting Fellow Visiting Fellow Visiting Fellow Staff Fellow Bio Lab Tech	LDB, NIDR LDB, NIDR LDB, NIDR LDB, NIDR LDB, NIDR LDB, NIDR
COOPERATING UNITS (if any) Shriner's Hospital, Portland, OR; Osaka University, Osaka, Japan; Wistar Institute Philadelphia, PA; HSP Research Institute, Osaka, Japan; Mark Sharp & Dohme Research Laboratories, NJ; Veterans Administration, CA.		
LAB/BRANCH Laboratory of Developmental Biology		
SECTION Molecular Biology Section		
INSTITUTE AND LOCATION NIDR, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 2.7	PROFESSIONAL: 1.7	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>           Transgenic animals created by introducing foreign DNA into the germ line provide insights into gene regulation, development, pathogenesis, and the treatment of disease. Analysis of the phenotypes of mice carrying mutations created by specific gene targeting using Embryonic Stem (ES) cells also provides insight into the biological role of genes whose functions have previously been only a matter of speculation. The purpose of this project is to create transgenic mice for studying the molecular basis of genetic and acquired diseases associated with connective tissues. These transgenic mice will also be useful in elucidating the role of these proteins in development. Genes for the basement membrane and cartilage components have been cloned and the exon-intron structure of these genes have been characterized. A transcriptional enhancer was identified in the first intron of the collagen II gene by DNA transfection into chondrocytes. Several deletion constructs of the enhancer and promoter of the collagen II gene were prepared using <math>\beta</math>-galactosidase as a reporter to delineate the enhancer sequence for its tissue and developmental stage-specific activity in mice. Some of the construct were injected into mouse oocytes and chimeric mice have been obtained for further characterization. Similar <math>\beta</math>-galactosidase reporter gene constructs have been prepared to study the enhancer of the collagen IV gene in vivo. Constructs for the expression of foreign genes in the cartilage of transgenic mice under the control of the promoter and enhancer of collagen II gene have been prepared to create animal models for human diseases such as arthritis. Mutations in the endogenous genes for basement membrane and cartilage proteins have been attempted by homologous recombination. Genes for collagen IV and perlecan were cloned and characterized by DNA sequencing. A segment of these genes were used to prepare gene targeting vectors for type IV and perlecan. These constructs were transfected into ES cells and screened for homologous recombination. Several ES lines have been established and been injected into mouse blastocysts to create chimeric animals.         </p>		



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 DE 00485-06 LDB
PERIOD COVERED October 1, 1993 to September 30, 1994		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Gene Regulation and Function of Basement Membrane		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Yamada, Yoshihiko	Section Chief LDB, NIDR
Others:	Burbelo, Peter D.	Senior Staff Fellow LDB, NIDR
	Utani, Atsushi	Visiting Fellow LDB, NIDR
	Nomizu, Motoyoshi	Visiting Associate LDB, NIDR
	Tanaka, Masahiko	Visiting Fellow LDB, NIDR
	Sugiyama, Satoru	Visiting Associate LDB, NIDR
	Takami, Hiro	Visiting Fellow LDB, NIDR
	Bernier, Susan	Special Volunteer LDB, NIDR
COOPERATING UNITS (if any) Max-Plank-Institute, Munich, Germany; Univ. of Pittsburgh, Pittsburgh, PA; Univ of Genova, Italy; MD Anderson Cancer Center, Houston, TX; INSERM U49, France; Univ of Iowa, Iowa City, IA.		
LAB/BRANCH Laboratory of Developmental Biology		
SECTION Molecular Biology Section		
INSTITUTE AND LOCATION NIDR, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.03	5.03	0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Basement membranes are thin sheets of extracellular matrix surrounding most tissues and play a critical role in tissue development, repair, and maintenance. The aim of this project is to understand the molecular mechanisms underlying the role of the basement membranes in these biological processes. Basement membranes contain a unique set of proteins, such as collagen IV, laminin, perlecan and nidogen/entactin. Laminin is a family of heteromeric glycoproteins specific in basement membranes and has a number of biological activities. Mouse laminin $\alpha 2$ chain was cloned and we determined its primary structure. Its tissue-specific expression was also studied by in situ and Northern hybridization, RNase protection assays and immunological analysis. Laminin $\alpha 2$ chain was significantly reduced in muscle, and peripheral nerves of dystrophic dy (dystrophia muscularis) mice. The laminin $\alpha 2$ chain gene was mapped to a region of mouse Chromosome 10 close to the dy locus, strongly suggesting the defect in the laminin $\alpha 2$ gene of dy mice. The molecular mechanism of laminin chain assembly was studied using recombinant chains in affinity chromatography and DNA transfection assays, and in vitro reconstitution. These studies revealed that a short specific sequence of each chain plays a critical role for the formation of an $\alpha$ -helical triple-stranded coiled coil structure of laminin. Conformation of these specific regions of laminin has also been studied by CD spectroscopy using synthetic peptides. Several active sequences for cell adhesion and migration have been identified in the G domain of the $\alpha 1$ chain. The $\alpha 1$ (IV) and $\alpha 2$ (IV) genes are separated by only 130 bp in a head-to-head orientation. It was found that these genes are regulated by several enhancer elements and chromatin structure surrounding the enhancer region.		





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 DE 00524-04 LDB												
PERIOD COVERED October 1, 1993 to September 30, 1994														
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Functions and Developmental Regulation of Matrix Receptors														
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Yamada, Kenneth M</td> <td style="width: 33%;">Chief</td> <td style="width: 33%;">LDB, NIDR</td> </tr> <tr> <td>Others: Brown, Karen E</td> <td>IRTA Fellow</td> <td>LDB, NIDR</td> </tr> <tr> <td>Lafrenie, Robert M</td> <td>Visiting Fellow</td> <td>LDB, NIDR</td> </tr> <tr> <td>Miyamoto, Shingo</td> <td>Visiting Fellow</td> <td>LDB, NIDR</td> </tr> </table>			PI: Yamada, Kenneth M	Chief	LDB, NIDR	Others: Brown, Karen E	IRTA Fellow	LDB, NIDR	Lafrenie, Robert M	Visiting Fellow	LDB, NIDR	Miyamoto, Shingo	Visiting Fellow	LDB, NIDR
PI: Yamada, Kenneth M	Chief	LDB, NIDR												
Others: Brown, Karen E	IRTA Fellow	LDB, NIDR												
Lafrenie, Robert M	Visiting Fellow	LDB, NIDR												
Miyamoto, Shingo	Visiting Fellow	LDB, NIDR												
COOPERATING UNITS (if any) University of Tennessee (Donaldson D); Walter Reed Army Institute of Research and CBER, FDA (Dhawan S); CNRS, France (Thiery JP); Kyoto University, Japan (Takeichi M); Univ. Turku, Finland (Larjava H); Univ. Helsinki, Finland (Thesleff I).														
LAB/BRANCH Laboratory of Developmental Biology														
SECTION Developmental Mechanisms and Disorders Section														
INSTITUTE AND LOCATION NIDR, NIH, Bethesda, Maryland 20892														
TOTAL STAFF YEARS: 1.54	PROFESSIONAL: 1.54	OTHER: 0												
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews														
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) <p>Integrins and other cell surface receptors for extracellular matrix proteins such as fibronectin function in embryonic development and wound healing. Our studies have demonstrated important roles for integrins in early development. Our recent findings suggest novel potential roles in tooth and salivary gland development, as well as in epithelial wound healing and in modulating protease production. In collaborative studies, a link between integrin binding and secretion of the metalloproteinase termed gelatinase B was discovered using an anti-integrin monoclonal antibody. Such functional linkages will be useful to characterize in order to understand how cells modulate protease-mediated changes in extracellular matrix. In tooth development, we identified unexpectedly rapid and precise changes in expression of mRNA for the <math>\beta 5</math> integrin in mouse molar rudiments, with large alternations of <math>\beta 5</math> mRNA expression between epithelium and mesenchyme occurring within a single day. Other integrins did not show this type of regulatory oscillation. Such novel integrin switching may have interesting regulatory roles during tooth development. Other studies are attempting to characterize the mutual cross-regulation of function of integrin and cadherin adhesion systems, as well as the regulation of integrin synthesis and differentiation in salivary gland cells by extracellular matrix. The roles of integrin ligand occupancy, aggregation, and a combination of these two events are being tested using natural ligands, soluble peptides, immobilized multivalent peptides, and monoclonal antibodies. Initial results indicate distinct roles for occupancy versus aggregation, and synergistic effects of the combination in controlling receptor location, signaling, and association with different classes of cytoskeletal molecules. These approaches provide novel tools for understanding how extracellular molecules regulate cellular functions. Because alterations in integrin function may contribute to a variety of human congenital defects and affect wound healing, these studies also provide an opportunity to identify new pathways as targets for potential therapy.</p>														



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00525-04 LDB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms and Regulation of Cell Adhesion, Migration, and Morphogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Yamada, Kenneth M	Chief	LDB, NIDR
Others:	Aota, Shin-ichi	Visiting Associate	LDB, NIDR
	Thomas, Linda A	Biologist	LDB, NIDR
	Lee, Chong-Chou	Visiting Fellow	LDB, NIDR
	Savagner, Pierre	Special Volunteer	LDB, NIDR
	Yamada, Susan S	Special Volunteer	LDB, NIDR

COOPERATING UNITS (if any)

CBER, FDA (Komoriya A); Dept. Anatomy, Univ. Pennsylvania (Lash J).

LAB/BRANCH

Laboratory of Developmental Biology

SECTION

Developmental Mechanisms and Disorders Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

4.75

PROFESSIONAL:

4.65

OTHER:

0.10

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Cell adhesion, migration, and morphogenesis are crucial events in craniofacial development, and errors in them can produce congenital anomalies. Similar processes appear to be important for normal adult wound repair. Molecules that mediate these processes and overall regulatory mechanisms are being characterized. Fibronectin is important for both morphogenesis and epithelial wound healing, e.g. for migration of craniofacial neural crest and keratinocytes. Regions of fibronectin essential for cell adhesion and migration were characterized in detail by site-directed mutagenesis and homology scanning approaches. The 5 amino acid sequence Pro-His-Ser-Arg-Asn was found to be essential for cell adhesion mediated by the  $\alpha 5 \beta 1$  fibronectin receptor. This information should facilitate the rational design of novel, specific bioadhesives and competitive inhibitors. Vitronectin is another major extracellular adhesion molecule. Analysis of its expression revealed striking mRNA localization in the spinal cord floor plate of mouse embryos. This structure has been identified as important for neuronal development, and vitronectin may be a novel effector molecule. Adhesion, migration, and morphogenesis are regulated by a variety of factors including cytokines and receptors such as the c-met proto-oncogene receptor. We have discovered a novel spliced version of this tyrosine kinase receptor in embryos and in a variety of tissues that deletes a key regulatory region involved in receptor regulation by protein kinase C. This and other receptors have been implicated in the interconversion of epithelia and mesenchyme in development. In a carcinoma system, FGF receptors have been implicated, and we found novel regulation of splicing and function of this family of receptors. We are also searching for novel proteins expressed during this interconversion by subtractive cloning. Over 30 cDNA clones have been identified and are being characterized to identify novel regulatory molecules. These studies should provide a molecular understanding of the regulation of these important but complex morphogenetic processes.







DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00559-03 LDB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biological Activities of HIV Proteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Kleinman, Hynda K.	Section Chief	LDB, NIDR
Others:	Roque, Eva M.	Biol Lab Tech	LDB, NIDR
	Johnson, Barbara A.	IRTA Fellow	LDB, NIDR
	Klotman, Paul E.	Special Expert	LDB, NIDR
	Yamada, Kenneth M.	Laboratory Chief	LDB, NIDR
	LaFrenie, Robert	Visiting Fellow	LDB, NIDR
	Subhash, Dhawan	Guest Researcher	LDB, NIDR

COOPERATING UNITS (if any)

St. Louis University, IL, Green M, Lowenstein M; NCI, NIH, Klotman M; NINCDS, NIH Lieberman D, Oldfield E.

LAB/BRANCH

Laboratory of Developmental Biology

SECTION

Cell Biology Section and Developmental Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.27

PROFESSIONAL:

1.77

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Patients with HIV infection who have AIDS are subject to an unexplained dementia even though virus is not observed in the brain. It is proposed that a soluble factor released from the virus may be affecting patients. We find that the HIV viral protein Tat promotes neural cell adhesion in vitro and blocks laminin-mediated process outgrowth. These events are mediated by a 90 kDa Tat receptor and were localized to a 9 amino acid sequence in Tat. Direct injection of Tat into the brains of rats caused impaired motor function and destruction of large amounts of brain tissue. High doses resulted in death. These data demonstrate that Tat has a strong effect on neural cells and suggest a possible mechanism to explain the neurologic changes and dementia observed in patients with AIDS. Infection of T cells with HIV-1 stimulated invasiveness through basement membrane, and synthesis of the 92 kDa type IV collagenase (gelatinase B). Monocyte infection by HIV-1 resulted in substantial increases in cell-cell adhesion mediated primarily by the integrin  $\alpha_L\beta_2$ . Increased adhesion of HIV-infected monocytes to a variety of endothelial cell types was followed by marked disruption of endothelial cell layers and increased vascular permeability, accompanied by increased expression of the 92 kDa metalloproteinase gelatinase B. This endothelial disruption was inhibited strongly by protease inhibitors including the TIMPs. These findings suggest the hypothesis that HIV-1 infection may activate blood monocytes to adhere to endothelium in aggregates, then to secrete increased amounts of protease activity resulting in endothelial disruption, thereby permitting invasion of HIV-infected cells into tissues. In preliminary studies, the HIV product Tat by itself induced secretion of the 92 kDa gelatinase B in dose-dependent fashion. These novel findings may provide a mechanism for metalloproteinase induction in HIV-infected cells, and they suggest that creative new approaches using specific metalloproteinase inhibitors to block tissue invasion might ultimately provide new insights into AIDS pathogenesis and preventive therapy.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00560-03 LDB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular and Functional Analysis of Membrane-Cytoskeletal Interactions

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Yamada, Kenneth M	Chief	LDB, NIDR
Others:	LaFlamme, Susan E	Senior Staff Fellow	LDB, NIDR
	Akiyama, Steven K	Research Chemist	LDB, NIDR
	Katz, Ben-Zion	Visiting Fellow	LDB, NIDR
	Miyamoto, Shingo	Special Volunteer	LDB, NIDR
	Yamada, Susan S.	Special Volunteer	LDB, NIDR

COOPERATING UNITS (if any)

FIC, NIH (Geiger B); Oncology, John Hopkins Hospital, Baltimore, MD, (Tucker R, Wilhide C); Dept. Immunopathology, Scripps Research Institute, La Jolla, CA, (Ginsberg M).

LAB/BRANCH

Laboratory of Developmental Biology

SECTION

Developmental Mechanisms and Disorders Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.35

PROFESSIONAL:

2.35

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The interface between the plasma membrane and the cytoplasm of cells is a crucial site for bidirectional information transfer between extracellular and intracellular processes as diverse as adhesion and tissue formation, cell and tissue movements, and the regulation of gene expression and cell growth. Coordination and spatial organization of cellular structures associated with this interface, such as adhesion sites and the intracellular actin-containing cytoskeleton, are thought to be crucial for key steps in embryonic development, wound healing, and differentiated tissue function. Integrins are the major class of receptors used by cells to interact with the extracellular matrix. Integrin receptors mediate cell adhesion, cell migration, and signaling from extracellular matrix molecules to the interior of cells. Integrins generally contain two relatively short cytoplasmic domains. The roles of these domains in controlling the location of receptors, signaling, and regulating cell behavior are being explored using molecular biology and biochemical methods. Functions of isolated domains are being tested using chimeric receptors containing a reporter domain consisting of a subunit of the interleukin-2 receptor and various integrin cytoplasmic tails. The  $\beta 1$ ,  $\beta 3$ , and to some extent  $\beta 5$  integrin cytoplasmic domains were found to contain sufficient information for the targeting of receptors to adhesion sites of cells. Further studies are characterizing the roles of integrin cytoplasmic domains in regulating messenger systems calcium concentration and pH. Overexpression of certain cytoplasmic domain chimeras produced a dominant negative phenotype. The  $\beta 1$  and  $\beta 3$  cytoplasmic domains inhibited cell spreading, cell migration, localization of endogenous fibronectin receptors to extracellular matrix contacts with fibronectin fibrils, fibronectin matrix assembly, and even signaling from the cytoplasm to external integrin receptors. The alternatively spliced  $\beta 3$  and the  $\alpha 5$  cytoplasmic domain chimeras had no effects. These studies demonstrate the central role of specific integrin cytoplasmic domains in cellular functions. They also provide novel tools for analyzing these processes and for determining the transmembrane spatial organization of adhesion/signaling complexes essential for coordinating the complex rearrangements and final organization of oral, facial, and other developing tissues. These interactions are also likely to be important for adult tissue repair.







DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 DE 00563-03 LDB
PERIOD COVERED October 1, 1993 to September 30, 1994		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Molecular Mechanisms of Cell-Substrate Interactions</b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Akiyama, Steven K.	Senior Staff Fellow LDB, NIDR
Others:	Yamada, Kenneth M.	Chief LDB, NIDR
	LaFlamme, Susan E.	Senior Staff Fellow LDB, NIDR
	Aota, Shin-ichi	Visiting Associate LDB, NIDR
	Kioka, Noriyuki	Visiting Fellow LDB, NIDR
	Torchia, Dennis	Section Chief BRB, NIDR
	Copie, Valerie	Visiting Fellow BRB, NIDR
	Tran, Michael N.	Biologist LDB, NIDR
COOPERATING UNITS (if any) Weizmann Institute of Science, Israel, Lider O; American Red Cross, MD, Ingham KC; University of Wisconsin, WI, Peters DP; Georgetown University Medical Center, DC Chen WT; University of Manchester, UK, Humphries M.		
LAB/BRANCH Laboratory of Developmental Biology		
SECTION Developmental Mechanisms and Disorders Section		
INSTITUTE AND LOCATION NIDR, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.95	2.45	0.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>             The adhesive glycoprotein fibronectin and its integrin receptors play important roles in embryonic development, wound healing and the progression of diseases such as cancer. Techniques involving protein biochemistry, monoclonal antibodies, molecular and cell biology, and physical biochemistry are used to elucidate molecular mechanisms of fibronectin-receptor interactions in order to understand their roles in cell adhesive processes with the long-term goals of producing novel bioadhesive substrates and developing the basis for the rational medical intervention in diseases involving abnormal cellular adhesion and migration. Integrin-mediated cell adhesion results in signal transduction in the form of tyrosine phosphorylation of a 125 kDa cytoplasmic focal adhesion kinase. The specific role of integrin intracellular domains in signal transduction has been examined using chimeric receptors, consisting of the extracellular and transmembrane domains of the non-signaling subunit of the interleukin-2 receptor, expressed in cultured human fibroblasts. Signaling was induced in cells expressing chimeric receptors containing the integrin <math>\beta 1</math>, <math>\beta 3</math>, and <math>\beta 5</math> intracellular domains but not in cells expressing chimeric receptors with no intracellular domain or containing either the <math>\alpha 5</math> or an alternatively-spliced form of the <math>\beta 3</math> intracellular domain. This result indicates that the information contained in specific integrin <math>\beta</math> intracellular domains is sufficient to stimulate signal transduction, integrin extracellular and transmembrane domains are not required for signal transduction, and alternative splicing can regulate the ability of integrins to participate in signal transduction. Tumor necrosis factor <math>\alpha</math> (TNF<math>\alpha</math>) binds to the amino-terminal region of fibronectin and enhances the adhesion of CD4<sup>+</sup> T cells. Recent results have indicated that the adhesion enhancing effect of fibronectin-bound TNF<math>\alpha</math> requires prior activation of the cells and both soluble and fibronectin-bound TNF<math>\alpha</math> synergizes with phorbol esters to trigger signal transduction in the form of tyrosine phosphorylation. The binding of a 70 kDa amino-terminal fragment of fibronectin to fibroblast monolayers has been found to require occupied <math>\alpha 5 \beta 1</math> integrins, with which it co-localizes in focal adhesions. Protease complexes called seprins have been identified on the leading edges of melanoma cells and in specialized cell surface protrusions called invadopodia. The expression of seprins appears to be a marker for highly invasive melanoma cells.           </p>		



PROJECT NUMBER Z01 DE 00563-03 LDB

"Professional Personnel, continued"

Do K

Biologist

LDB, NIDR





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00034-26 LI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms of Histamine Release

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Siraganian, Reuben	Chief, RASTS	LI	NIDR
Bader, Greta	Biologist	LI	NIDR
Berenstein, Elsa	Microbiologist	LI	NIDR
Bhattacharyya, Siba	Visiting Associate	LI	NIDR
Hamawy, Majed	Staff Fellow	LI	NIDR
Hook, William	Guest Researcher	LI	NIDR
Kihara, Hidetoshi	Visiting Fellow	LI	NIDR
Kimura, Teruaki	Visiting Fellow	LI	NIDR
Mergenhagen, Stephan E.	Chief, LI	LI	NIDR continued.

COOPERATING UNITS (if any)

National Cancer Institute, NIH; Sloan Kettering Institute, NY; Weizman Institute, Israel; Harvard Medical School, MA

LAB/BRANCH

Laboratory of Immunology

SECTION

Receptors and Signal Transduction Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

8.08

PROFESSIONAL:

7.08

OTHER:

1.00

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Histamine release from mast cells and basophils is being studied as an immunological mechanism involved in inflammation. The activation of these cells by immune cell surface receptors is also a model for signal transduction and cell secretion. Besides cell surface receptors, the cells can be activated by other secretagogues such as the calcium ionophore A23187. The emphasis the last few years has been in understanding the role of protein tyrosine phosphorylations in the signalling. The cultured rat basophilic leukemia cells are used as one of the main models for these studies.



Z01DE00034-26 LI

Professional Personnel, continued:

Swieter, Mark	Senior Staff Fellow	LI	NIDR
Tomlinson, Nicola	Visiting Fellow	LI	NIDR





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00046-23 LI

PERIOD COVERED

October 01, 1993 - September 30, 1994

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Normal and Aberrant Mechanisms of Inflammation, Repair, and Regeneration

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Sharon M. Wahl	Chief, CIS	LI, NIDR
Marielle Christ	Visiting Fellow	LI, NIDR
Nandita Chopra	Biologist	LI, NIDR
Keith Hines	PRAT Fellow	LI, NIDR
Nancy Francis	Special Expert	LI, NIDR
Mary Slater-Venkata	Biologist	LI, NIDR
Joseph Bryant	Veterinarian	ACU, NIDR

COOPERATING UNITS (if any)

Ashok Kulkarni, Stefan Karlsson, NIMH; J.M. Ward, NCI; J. McCarthy, L. Furcht, Univ. Minnesota; R. Redman, VA Hospital.

LAB/BRANCH

Laboratory of Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

3.75

PROFESSIONAL:

2.7

OTHER:

1.05

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Transforming growth factor  $\beta$  (TGF- $\beta$ ) is a multifunctional cytokine involved in development, repair and regeneration. To explore its role in immune and inflammatory processes, mice deficient in a functional TGF- $\beta$ 1 gene [TGF- $\beta$ 1(-/-)] were studied. Although initially appearing normal, likely due to maternal transfer of TGF- $\beta$ 1, within 10-20 days after birth, the TGF- $\beta$ (-/-) mice began to waste and die. Histopathology revealed dramatic numbers of mononuclear leukocytes (MNL) in vital organs, leading to organ failure and death. Phenotypically, the TGF- $\beta$ 1(-/-) lymphoid cells expressed enhanced differentiation antigens proliferating cells, with increased IL-2 and IL-2R mRNA. In contrast with the activation profile of TGF- $\beta$ 1(-/-) lymphoid cells in vivo, mitogen challenge of these cells in vitro revealed suppressed proliferation and a defect in inducible IL-2. Moreover, the addition of rIL-2 restored the deficient mitogen-induced proliferation. The mechanism leading to T cell anergy remains unclear; however, these data confirm the essential role for TGF- $\beta$ 1 in maintaining normal immune function. Consistent with the increased leukocyte infiltration of tissues, MNL from symptomatic TGF- $\beta$ 1(-/-) mice, exhibited increased adhesion to extracellular matrix and endothelial cells. Incubation of TGF- $\beta$ 1(-/-) MNL with synthetic peptides corresponding to cell- and heparin-binding sequences of fibronectin (FN) significantly attenuated binding to FN and endothelial cells in vitro. Based on these observations, mice were treated with the FN peptides in an attempt to rescue them from tissue inflammation and cardiopulmonary failure. Daily injections of four synthetic FN peptides that interact with  $\beta$ 1-integrins and/or cell surface proteoglycans blocked the massive infiltration of MNL into the heart and lungs, and also moderated the lethal wasting syndrome. The wasting syndrome may also be related to a nutritional deficit resulting from defective dentition, and studies are in progress to delineate the role of TGF- $\beta$  in the development of the oral cavity, as well as in other developmental and immunological processes.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00199-18 LI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

In Vitro Studies of Secretory Cell Structure and Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Oliver, Constance	Guest Research Biologist	LI	NIDR
Swaim, William D.	IRTA Fellow	LI	NIDR
Siraganian, Reuben P.	Chief, RASTS	LI	NIDR
Waters, Judith F.	Biologist	LI	NIDR
Weedon, Lynda L.	Biologist	LI	NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunology

SECTION

Receptors and Signal Transduction Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

3.98

PROFESSIONAL:

1.98

OTHER:

2.00

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The secretory process in several cell types is being investigated. The rat basophilic leukemia cell line (RBL-2H3) and other cultured cells are used to study various aspects of endocytic and secretory processes. Emphasis is on the use of morphological, cytochemical and biochemical characterizations in these cultured cells. Events involved in receptor activation and signal transduction are being investigated, as well as endocytic and secretory pathways. During the last year emphasis has been on the study of the biochemical changes induced by the binding of a monoclonal antibody to a cell surface ganglioside.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00290-15 LI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Production of Hybridomas

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Siraganian, Reuben P.	Chief, RASTS	LI	NIDR
Berenstein, Elsa H.	Microbiologist	LI	NIDR
Fischler, Cynthia	Microbiologist	LI	NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunology

SECTION

Receptors and Signal Transduction Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.75

PROFESSIONAL:

.75

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Hybridomas are being produced which secrete monoclonal antibodies to defined antigen specificity. In the past hybridomas have been selected that recognize the high affinity IgE receptor, its components and associated cell surface proteins that are involved in signal transduction. Monoclonal antibodies to several other cell surface proteins have also been selected. During the last year monoclonal antibodies were produced that recognize phosphorylated proteins.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00424-09 LI

PERIOD COVERED

October 1, 1993 - September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Analysis of Monocyte Phenotype and Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Nancy McCartney-Francis	Expert	LI NIDR
Sharon Wahl	Chief, Cellular Immunology	LI NIDR
Diane Mizel	Chemist	LI NIDR

COOPERATING UNITS (if any)

Hank Towle, DDS, Chief of Periodontics, Naval Medical Center; Wayne Leadbetter, M.D., Orthopedic Center; Ashok Kulkarni, Ph.D., NINDS; Pam Manning, Ph.D., Searle Research and Development, Monsanto; Uros Skaleric, D.D.S.,

LAB/BRANCH

Laboratory of Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.8

PROFESSIONAL:

1.2

OTHER:

0.6

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objective of this research program is to define the phenotypic and functional properties of monocytes and the molecular mechanisms that regulate the inflammatory process. One effector molecule that has been implicated as a mediator of immune and inflammatory responses is nitric oxide (NO), a toxic radical gas produced during the metabolism of L-arginine by NO synthase (NOS). Development of erosive arthritis in rats following the injection of streptococcal cell walls (SCW) is accompanied by induction of mRNA and protein for inducible NOS (iNOS) and production of NO both in synovial tissue from the inflamed joint and blood mononuclear cells and plasma. SCW and cytokines trigger transcription of this important enzyme. Further, daily systemic administration of an inhibitor of NOS, N<sup>G</sup>-monomethyl-L-arginine (NMMA), prevented tissue damage and joint destruction. These studies implicate the NO pathway in the development of polyarthritis and the use of NOS inhibitors in the treatment of arthropathies and other inflammatory diseases. Additional NOS inhibitors including aminoguanidine, which is specific for iNOS, hemoglobin, which scavenges NO, and immunosuppressive cytokines, including transforming growth factor beta (TGF- $\beta$ ) and interleukin-4 (IL-4) are also effective inhibitors of SCW-induced inflammation. The demonstrated involvement of the NO pathway in arthritic rats suggests that NO contributes to the development of certain human inflammatory diseases, including rheumatoid arthritis and periodontal disease. Elevated nitrite levels in synovial and gingival fluids correlate with degree of inflammation in the tissue and iNOS protein can be detected in inflamed synovial and gingival tissue. Continued delineation of the cellular source(s) and molecular regulation of the specific NOS (iNOS or cNOS) involved in the pathologic overproduction of NO will facilitate identification of the most effective NOS inhibitors for diagnosis and treatment of these and other inflammatory and immune mediated disorders.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 DE00441-08 LI

PERIOD COVERED

October 01, 1993 - September 30, 1994

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Regulation of Chronic Immune/Inflammatory Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: John Zagorski	Staff Fellow	LI, NIDR
Tomozumi Imamichi	Fogarty Fellow	LI, NIDR
Sharon Wahl	Chief, CIS	LI, NIDR
Nancy Francis	Special Expert	LI, NIDR
Keith Hines	PRAT Fellow	LI, NIDR

COOPERATING UNITS (if any)

J.B. McCarthy, University of Minnesota; C. Geczy, Australia; W. Leadbetter, Orthopedic Center; U. Skaleric, Slovenia; M.E. Brandes, Proctor & Gramble

LAB/BRANCH

Laboratory of Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

2.3

PROFESSIONAL:

2.3

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Administration of group A streptococcal cell wall (SCW) peptidoglycan-polysaccharide complexes induces arthritis, liver fibrosis, and spleen cell anergy in genetically susceptible rodents. Studies continue on determining the genetic basis for susceptibility utilizing a cDNA subtraction library from genetically resistant (F344/N) and susceptible (LEW/N) rat strains. In addition, this model provides a system to explore all phases of an immune response and the consequences of its dysregulation. Increased leukocyte adhesion to endothelial cells and extracellular matrix is associated with the evolution of synovial inflammation. Leukocytes from animals receiving arthrophathic doses of SCW demonstrate increased integrin mRNA expression and enhanced adhesion to fibronectin and/or endothelial cells. To determine whether this augmented adhesion was causal in the development of synovitis, peptides synthesized from fibronectin domains which inhibited leukocyte adhesion in vitro were administered to arthritic animals. Not only were peptides containing either the RGD or CS-1 cell-binding domains inhibitory, but three peptides from the carboxy-terminal 33kD heparin-binding domain of fibronectin were also found to significantly inhibit arthritis. Based on these data, which are the first to explore the therapeutic potential of heparin-binding fibronectin peptides in chronic inflammation, it appears that antagonism of cellular adhesion and recruitment by these peptides may provide an important mechanism for modulating the multi-step adhesion process and attenuating aberrant inflammatory responses. Chemokines are also pivotal in the recruitment process, and the molecular, biochemical, and functional identification of chemokines in the inflamed tissues prompted the use of specific antagonists to block activity and determine their pathologic relevance. Continued definition of these experimental pathways directs our analysis of human chronic inflammatory disorders including arthritis, injury-associated disorders and periodontitis.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00456-07 LI

PERIOD COVERED

October 01, 1993 - September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Signal Transduction in the Monocyte/Macrophage

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Larry L. Wahl	Senior Scientist	LI, NIDR
Prema Mertz	Senior Staff Fellow	LI, NIDR
Kevin McCluskey	Chemist	LI, NIDR
Yahong Zhang	Special Volunteer	LI, NIDR

COOPERATING UNITS (if any)

I. Katona, USUHS; W. Stetler-Stevenson, NCI; I. Horak, NCI; S. Dhawan, FDA;  
D. DeWitt, Michigan State University

LAB/BRANCH

Laboratory of Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

3.83

PROFESSIONAL:

2.0

OTHER:

1.83

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The major emphasis of this project is on the biochemical events involved in the PGE2-cAMP dependent signal transduction in the monocyte that leads to the production of matrix metalloproteinases (MMP). Recent studies have examined the mechanism by which cytokines may modulate MMP synthesis. The Th2 derived cytokines, IL-4 and IL-10, were shown to inhibit the induction of prostaglandin H synthase-2 (PGHS-2) but had no effect on the endogenous PGHS-1 expression in monocytes. In vivo administration of IL-4 to cancer patients was also shown to suppress the induction of PGHS-2 in their peripheral blood monocytes. In vitro studies demonstrated that inhibition of PGHS-2 occurred at the transcriptional level and resulted in the suppression of PGE2, thus blocking the PGE2 dependent production of interstitial collagenase and 92-kD type IV collagenase (gelatinase B) as demonstrated at the mRNA and protein level. This inhibition was reversed by the addition of PGE2 or Bt2cAMP. In contrast to IL-4 and IL-10, Th1 derived cytokines, such as IL-2, and the colony stimulating factors (IL-3, M-CSF, and GM-CSF), enhanced mRNA and protein levels of PGHS-2, interstitial collagenase and gelatinase B by LPS stimulated monocytes. Additional studies have focussed on the effect of laminin derived peptides containing the YIGSR and SIKVAV amino acid sequences on monocyte MMP production. These peptides, in contrast to the intact laminin molecule, stimulated or enhanced monocyte PGE2 and MMP synthesis demonstrating that laminin fragments may play an important role in regulating connective tissue turnover. Studies conducted in collaboration with scientists at FDA have revealed that interferon- $\gamma$  suppresses HIV-induced MMP production by monocytes. We have also shown that this cytokine induces resistance in monocytes to HIV infection.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00513-05 LI

PERIOD COVERED

October 01, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Monocytes in AIDS and as Targets for Antiviral Therapy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Sharon Wahl	Chief, Cellular Immunology Section	LI, NIDR
Lauren Flores	PRAT Fellow	LI, NIDR
Tessie McNeely	Sr. Staff Fellow (CRADA)	LI, NIDR
Marian Dealy	Biologist (CRADA)	LI, NIDR

COOPERATING UNITS (if any)

S. Eisenberg, and R. Thompson, Synergen, Boulder, CO; Jan Orentein, GWU; J.B. McCarthy, Univ. Minnesota; P.D. Smith, Univ. Alabama; Ed Janoff, Univ. Minnesota

LAB/BRANCH

Laboratory of Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.1

PROFESSIONAL:

0.1

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Monocytes and macrophages express CD4 on their surface, are targets for HIV-1, and may serve as a reservoir for the virus. Ongoing studies focus on the role this population plays in the progression of HIV-1 disease and as targets for antiviral therapy. Infection of adherent primary monocytes with HIV-1 is significantly suppressed in the presence of human saliva. By reverse transcriptase levels, saliva, although present for only 1 hour during viral exposure, inhibited HIV-1 for three weeks postinfection. Salivary antiviral activity was identified in the soluble fraction, and to determine the factor(s) responsible, individual saliva proteins were examined. Of those proteins examined, only secretory leukocyte protease inhibitor (SLPI) was found to possess anti-HIV activity at physiological concentrations. SLPI anti-HIV activity was dose dependent with maximal inhibition at 1-10 mg/ml (>90% inhibition of RT activity). SLPI also partially inhibited HIV-1 infection in proliferating T cells. Significantly, equivalent anti-HIV activity was observed when SLPI was preincubated with monocytes, then washed away, before addition of HIV. This anti-HIV activity was not due to cellular toxicity, or down-regulation of the CD4 antigen. Ultrastructural analysis confirmed the RT assays, demonstrating not only reduced numbers of infected cells, but also decreased viral load in cells containing HIV-1. These data indicate that SLPI has antiviral activity and contributes to the important antiviral activity of saliva and the infrequent oral transmission of HIV-1. Recent evidence suggests a role for adhesion molecules (integrins) in viral infection. Viral infection also modulates adhesion receptors on monocytes and lymphocytes which influences trafficking, signal transduction and cellular function. In this regard, synthetic peptides which bind to and block integrin binding sites appear to impair HIV-1 infection of monocytes in vitro. Continued analysis of HIV-infected monocyte phenotype and function will reveal additional targets for intervention.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00561-03 LI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Taste and Smell

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Ryba, Nicholas	Visiting Associate	LI	NIDR
Hoon, Mark	Visiting Fellow	LI	NIDR
Siraganian, Reuben P.	Chief, RASTS	LI	NIDR
Wu, Youmei	Visiting Fellow	LI	NIDR

COOPERATING UNITS (if any)

Dr. R. Tirindelli, University of Parma, Italy  
Dr. J. Northup, (See MS)  
Dr. R. F. Margolskee, Hoffman La Roche, Nutley, NJ

LAB/BRANCH

Laboratory of Immunology

SECTION

Receptors and Signal Transduction Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda Maryland 20892

TOTAL STAFF YEARS:

3.20

PROFESSIONAL:

3.20

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Molecular mechanisms of signal reception and transduction in taste and smell are being studied. The G-protein  $\alpha$ -subunit that appears to function in taste discrimination has been expressed and its functional activity compared with that of other  $\alpha$ -subunits in reconstitution experiments. The gene for this protein is being studied to address questions about taste specific gene expression. The expression of a novel G-protein  $\gamma$ -subunit from the developing neurons of the olfactory and vomeronasal epithelia has been characterized in detail; its function in olfactory neurogenesis is being investigated. The gene for this subunit is being studied to determine whether particular controlling elements are responsible for its expression pattern. Another novel G-protein  $\gamma$ -subunit that appears to be involved in olfactory signal transduction has been purified; it is being sequenced for cloning; functional studies of the purified protein are also being carried out.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00609-01 LI

PERIOD COVERED

October 01, 1993 - September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Immune Responses in Mucosal Tissues

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Anna George	Visiting Associate	LI, NIDR
Stephan Mergenhagen	Chief, LI	LI, NIDR
Nandita Chopra	Biologist	LI, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.75

PROFESSIONAL:

1.50

OTHER:

.25

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Mice  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objective of this newly initiated research proposal is to study the regulation of B and T cell responses in mucosal tissues. There are two specific aims. The first is to look at factors that are responsible for the predominant switching of mucosal B cells to IgA. A system has been set up in which small numbers of purified naive B cells are stimulated allogeneically with T cells and APCs isolated from mucosal or non-mucosal lymphoid organs. Culture supernatants are assayed for the presence of IgA, and the goal is to identify specific cells or cytokines that mediate isotype switching to IgA. The second aim is to compare responses of mucosal and non-mucosal T cells in mice that have been immunized either orally or intraperitoneally with an enteric pathogen. Whether Th1 or Th2 T cells predominate in Peyer's patches and mesenteric lymph nodes seems to depend on the immunization protocol used. The results have implications for the designing of mucosal vaccines.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00610-01 LI

PERIOD COVERED

October 01, 1993 - September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Monocyte Signalling Pathways in Apoptosis and Activation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Hong-Duck Um  
Sharon Wahl

Fogarty Fellow  
Chief, CIS

LI, NIDR  
LI, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.1

PROFESSIONAL:

1.1

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Apoptosis is an important mechanism whereby homeostasis of monocytes is achieved. In understanding the regulation of monocyte death, we have previously identified extracellular stimuli which can act as either inducers or inhibitors of apoptosis in vitro. Whereas serum depletion and IL-4 drive apoptosis, pro-inflammatory mediators including LPS, IL-1 $\beta$ , and TNF- $\alpha$  block this pathway. Additionally, HIV-1 infection has been associated with initiation of the apoptosis pathway. With growing evidence that Fas, a cell surface glycoprotein, can mediate an apoptotic signal in a number of cell lines, we have investigated the possible expression and subsequent function of Fas in human monocytes. The data indicate that Fas is highly expressed on monocytes and down-regulated by  $\gamma$ IFN, a cytokine that can protect cells from death. Stimulation through the Fas receptor by a specific antibody (anti-Fas) induces apoptosis of monocytes which otherwise have a prolonged life-span. Thus, Fas may be an important mechanism for controlling the number of monocytes that participate in host immune responses. Experiments were also designed to investigate the intracellular components involved in the death pathway. We have found that PKC inhibitors block the ability of LPS, IL-1 $\beta$ , or TNF $\alpha$  to rescue cells from apoptosis. Consistent with this finding, stimulation of PKC by PMA resulted in survival of cells, suggesting a critical role of PKC in the regulation of monocyte death. The protective effect of PMA was completely abolished by the co-presence of tyrosine kinase inhibitors. These data suggest that a tyrosine kinase(s) functions downstream from PKC. Unravelling these signalling pathways is essential to understanding what regulates whether a cell lives or dies in physiologic or pathologic conditions.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00043-24 LME

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Physiological and Genetic Studies of Oral Microorganisms

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Donkersloot, Jacob A., Research Microbiologist, LME, NIDR

Others: Thompson, John, Visiting Scientist, LME, NIDR

Robrish, Stanley A, Research Microbiologist, LME, NIDR

Pikis, Andreas, Staff Fellow, LME, NIDR

Harr, Robert, J, Biolaboratory Technician, LME, NIDR

COOPERATING UNITS (if any)

David W. Rice, Department of Molecular Biology and Biotechnology, University of Sheffield, Sheffield, United Kingdom

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Bacterial Toxins and Vaccines Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

0.6

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

It has recently been found (Robrish et al (1994) J. Bacteriol. 176:3250) that *Fusobacterium mortiferum* (ATCC 25557) metabolizes maltose via an inducible phosphoenolpyruvate:maltose phosphotransferase system. The maltose 6-phosphate (M6P) formed from this group translocation is subsequently converted to glucose 6-phosphate and glucose by the enzyme M6P hydrolase (J. Thompson et al., manuscript in preparation). The objective of this project has been to characterize the substrate specificity and regulation of the *F. mortiferum* maltose system (which comprises the translocation and hydrolysis activities, and any ancillary regulatory proteins) via the isolation and characterization of mutants. Initially, maltose-negative mutants were sought. One such mutant was obtained (FM12) that did not grow on maltose, isomaltose and palatinose, which indicated that these latter two  $\alpha$ -glucosides are also substrates of the maltose system. Subsequent enzyme assays showed that FM12 was deficient in M6P hydrolase activity. SDS-polyacrylamide gel electrophoresis revealed that this mutant expressed a protein that was induced like M6P hydrolase and that had the size of M6P hydrolase. Together, these data indicate that the maltose-negative phenotype of FM12 is associated with expression of a (virtually) full-length, but non-functional, M6P hydrolase. With respect to the specificity and regulation of the *F. mortiferum* maltose system, results indicate that this system has an unusually broad substrate specificity for  $\alpha$ -glucosides, but that induction is much more specific, because only maltose and (especially) isomaltose are efficient inducers. Evidence has also been obtained which suggests that the failure of non-induced *F. mortiferum* cells to proliferate on certain  $\alpha$ -glucosides (e.g. trehalose) is due to the inability of the cell to synthesize the appropriate intracellular inducer to (either positively or negatively) interact with a regulatory protein to affect transcription of one or more components of the maltose system. To support this hypothesis, phenotypically constitutive mutants have been isolated which grew (without a lag) on maltitol,  $\alpha$ -methyl glucoside, trehalose, turanose, and palatinose. In contrast to the parent, glucose-grown cells of these mutants also expressed high levels of maltose system activity (as measured by the formation of p-nitrophenol from p-nitrophenyl  $\alpha$ -glucoside).



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00254-17 LME

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Microbial Antigens Associated with Specific Adherence

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Cisar, John O., Research Microbiologist, LME, NIDR

Others: Hsu, S. Dana, Microbiologist, LME, NIDR  
Takahashi, Yukihiro, Visiting Fellow, LME, NIDR  
Sandberg, Ann L, Section Chief, LME, NIDR

COOPERATING UNITS (if any)

University of Florida; University of Maryland; University of Aarhus, Denmark; Georgetown University, University of Texas, San Antonio

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Microbial Receptors and Pathogenesis Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

2.5

PROFESSIONAL:

1.5

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Gram positive bacteria, primarily streptococci and actinomyces, initiate oral microbial colonization, subsequent dental plaque formation and gingival inflammation. Extensive studies have extended the recent reclassification of viridans streptococci to include results from *in vitro* assays for a number of well documented bacterial adhesin and receptor activities. The findings provide an improved perspective on the possible involvement of different specific interactions in colonization. The coaggregation properties of streptococci with actinomyces may be associated with different phases of colonization and plaque development. Thus, with adult isolates, virtually all streptococci that gave lactose resistant coaggregations with actinomyces were strains of *S. gordonii* or *S. anginosus*, species that occur primarily in mature supragingival and subgingival plaque, respectively. In contrast, streptococci that participated in lactose sensitive coaggregations with actinomyces included each of 11 *S. oralis* strains, a species involved in primary colonization, and 13 of 60 strains placed in other species. To facilitate studies of structure and function, cell wall polysaccharides have been purified from 21 of the 24 streptococcal strains that participated in lactose sensitive coaggregations with *Actinomyces* spp. Each polysaccharide was linear and formed by a characteristic hexa- or heptasaccharide subunit linked end-to-end by phosphodiester bonds. A common feature of each oligosaccharide subunit involved the presence of Galf( $\beta$ 1-6) which was followed by GalNAc( $\beta$ 1-3)Gal with certain strains or Gal( $\beta$ 1-3)GalNAc with others. Significantly, the latter disaccharides occur commonly in various glycoconjugates of host origin and have been previously identified as potential receptors for *Actinomyces* spp. lectins. Whereas the presence of these structures within the different polysaccharides accounts for bacterial lectin recognition, other structural features not shared with host cell glycoconjugates account for the reactions of specific antipolysaccharide antibodies. The dramatic difference between lectin and immunological recognition of these molecules may reflect low immunogenicity of lectin receptor structures within the polysaccharide as a result of their mimicry of host glycoconjugates. Ecological implications of these results are being further explored.







DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00273-16 LME

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cell-Cell Interactions Between Oral Actinomyces and Other Bacteria

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Kolenbrander, Paul, Research Microbiologist, LME, NIDR

Others: Andersen, Roxanna, Microbiologist, LME, NIDR  
Clemans, Daniel, IRTA Fellow, LME, NIDR  
Klier, Christiane, Visiting Fellow, LME, NIDR  
Whittaker, Catherine, Visiting Fellow, LME, NIDR  
London, Jack, Research Microbiologist, LME, NIDR

COOPERATING UNITS (if any)

Dr. P. Handley, University of Manchester, England; Dr. N. Ganeshkumar, Forsyth Dental Center, Boston, MA; Dr. F. Neuhaus, Northwestern University, Evanston, IL; Dr. H. Jenkinson, University of Otago, New Zealand; Dr. A. Callaway, Mainz, Germany

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Clinical Microbiology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

5.41

PROFESSIONAL:

4.0

OTHER:

1.41

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The focus of our research program is to understand the role of coaggregation in bacterial accretion of early colonizing bacteria on a clean tooth surface. The primary colonizers include actinomyces and streptococci. The 34.8-kDa lipoprotein surface-adhesin, ScaA, from *Streptococcus gordonii* PK488 is encoded as part of a putative ATP-binding cassette operon similar to the operons encoding the binding-protein dependent transport system of Gram-negative bacteria and the binding lipoprotein dependent transport systems of Gram-positive bacteria. ScaA mediates coaggregation between the streptococcus and its actinomyces partner cell. ScaA probably is anchored in the cell membrane through its lipid moiety, while its receptor-recognizing binding site is exposed to the environment. Streptococci also coaggregate with other genetically distinct streptococci. Transposon mutagenesis has been used to identify two coaggregation-relevant genes in *Streptococcus gordonii* DLI. The DNA sequence flanking one transposon indicates greater than 50% identity with genes involved in lipoteichoic acid synthesis in closely related *Lactobacillus casei*. The sequence flanking the other transposon has no homology to any sequences in the gene bank, but the coaggregation-defective (COG-) mutant cell has lost a 100-kDa protein from its surface. This protein is also absent in surface preparations from six spontaneous COG-mutants, and it is proposed that the protein is an adhesin mediating intragenetic coaggregation. *Actinomyces* serovar WVA963 strain PK1259 exhibits only lactose-inhibitable coaggregations with streptococci. Spontaneous COG- mutants were isolated and surface preparations from parent and mutant cells revealed a 95-kDa protein as the putative lactose-sensitive adhesin which mediates coaggregation with streptococci. The long range goal of these studies, collectively, is to elucidate the molecular mechanisms responsible for bacterial colonization in the human oral ecosystem.



Z01 DE00273-16 LME

Cell-Cell Interaction Between Oral Actinomyces and Other Bacteria

Professional Personnel, continued

Roble, Arlene	Summer Irta	LME, NIDR
Pokai, Sandra	Special Volunteer	LME, NIDR





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00341-13 LME

PERIOD COVERED

October 1, 1993 - September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Sugar Transport and Metabolism in Lactic Acid and Oral Bacteria

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Thompson, John, Visiting Scientist, LME, NIDR

Others: Robrish, Stanley A., Research Microbiologist, LME, NIDR  
Gentry-Weeks, Claudia R., Senior Staff Fellow, LME, NIDR

COOPERATING UNITS (if any)

Fales, Henry M., Lab Chief, LCB, NHLRI; Nguyen, Nga Y., Biologist, CBER, FDA;  
Davidson, Barrie E., Prof. Biochemistry, University of Melbourne

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Bacterial Toxins and Vaccines Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.9

PROFESSIONAL:

0.9

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The products of fermentation of carbohydrates by lactic acid bacteria, and of amino acids by *Fusobacteria*, comprise organic acids and potentially toxic sulfur-containing derivatives. These end-products of microbial metabolism are believed to be causative or contributory agents to the etiology of various oral diseases, including: dental caries, gingivitis and periodontitis. In this program we are attempting to define the biochemical and genetic factors responsible for regulation of these fermentative processes in pathogenic oral microorganisms. Major accomplishments toward this goal in the past year include: 1) The purification to homogeneity and characterization of triosephosphate isomerase from *Lactococcus lactis*, and cloning and sequence analysis of the chromosomally-encoded *tpi* gene.; 2) Biosynthesis, preparative isolation, and physiochemical characterization of maltose-6-phosphate from *Fusobacterium mortiferum*.; 3) Purification to homogeneity, characterization and N-terminal sequence determination of maltose-6P hydrolase from *F. mortiferum*.; and 4) Site-directed mutagenesis and identification of conformationally, and catalytically important lysyl and cysteinyl residues of B-cystathionase. Results from this research program have been published in three articles in international peer-reviewed journals. A chapter describing our studies in relation to regulation of sugar metabolism by lactic acid bacteria has been published in the French treatise, "Bacteries Lactiques", 1994.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00382-11 LME

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Growth and Metabolism of Oral Microorganisms

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Robrish, Stanley A., Research Microbiologist, LME, NIDR

Others: Thompson, John, Visiting Scientist, LME, NIDR

Gentry-Weeks, Claudia, Senior Staff Fellow, LME, NIDR

Donkersloot, Jacob, Research Microbiologist, LME, NIDR

COOPERATING UNITS (if any)

Fales, Henry, Laboratory Chief, LBC, NHLBI

Nguyen, N., Biologist, CBER, FDA

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Bacterial Toxins and Vaccines Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1

PROFESSIONAL:

1

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Maltose 6-phosphate, a compound unavailable commercially, has been made in milligram amounts using the phosphoenolpyruvate (PEP)-dependent phosphotransferase (maltose PTS) system resident in *Fusobacterium mortiferum* ATCC 25557. The proposed structure of maltose-6-P, the phosphate on carbon-6 of the non-reducing glucose moiety of the disaccharide, was confirmed by mass spectral analysis comparing the spectrum of the putative compound with those from sugar phosphate standards. Maltose use from a buffered medium by *F. mortiferum* was obligately anaerobic and ceased immediately when air was introduced. Maltose-6-phosphate was formed aerobically when permeabilized cells of *F. mortiferum* were incubated with maltose and PEP. The maltose-6-P could be hydrolyzed if the assay system was incubated with dithiothreitol [DTT] showing the hydrolase activity was preserved by reduced conditions. Maltose-6-P was hydrolyzed to equimolar amounts of glucose-6-P and glucose by a sonic extract whose hydrolase activity was preserved with DTT or exclusion of air. Maltose grown *F. mortiferum* used alpha-glucosides and cultures of the organism grown on alpha-glucosides used maltose suggesting a common transport system for these compounds. P-nitrophenyl alpha-D-glucoside [PNPG] was hydrolyzed, dependent on PEP, by permeabilized, maltose grown *F. mortiferum*. Permeabilized cells, grown on a variety of different alpha glucosides, could also hydrolyze PNPG, dependent on PEP in the assay, suggesting the maltose transport system was induced by, and used, alpha-glucosides. The phosphate derivative of PNPG was made [PNPGP] which was hydrolyzed by an activity identified as the maltose-6-P hydrolase. The intensity of a 52 KD band, seen in a gel, was directly related to PNPGP hydrolase activity assayed in sonic extracts of *F. mortiferum* grown on a variety of sugars. The 52 KD protein, identified as the maltose-6-P hydrolase, has been purified to homogeneity from maltose grown *F. mortiferum* using PNPGP hydrolysis as the assay.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00454-08 LME

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Surface Molecules in Colonization and Biological mimicry

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: London, Jack P., Section Chief, LME, NIDR

Others: Bouma, Carolyn, Staff Fellow, LME, NIDR

Kolenbrander, Paul E., Research Microbiologist, LME, NIDR

Lunsford, R. Dwayne, Senior Staff Fellow, LME, NIDR

COOPERATING UNITS (if any)

Dr. A. Hand, University of Connecticut; Dr. J. Manch-Citron, University of Missouri; and  
Dr. I. Weiss, Tel Aviv University, Tel Aviv Israel

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Clinical Microbiology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The importance of adhesins and their support structures in infectious processes has been well documented for a number of pathogenic bacteria. Similar principles have been established for the colonization of certain Gram negative oral bacteria by identifying and characterizing their lectin and non lectin adhesins. The structure of the *Prevotella loescheii* galactoside-specific adhesin has been deduced from its gene sequence. Recent studies have focused on the unusual mode of translation of adhesin mRNA; it occurs via the only frameshifting hop reported in prokaryotes. The contribution of the important elements of the hop, the shift region, stem loop and pseudoknot, are currently being assessed in a  $\beta$ -galactosidase  $\alpha$ -subunit construct containing the entire hop region. The efficient rate of read-through in the *Escherichia coli* model system and the precision with which the gene product is measured has simplified the problem of isolating effects produced by targeted mutations in each of the elements. In conjunction with these studies, the mRNA region at which the ribosome resumes translation has been narrowed to 12 codons. Data suggest that a 27 nucleotides region is not translated during the hop maneuver. The non-lectin, actinomyces-specific adhesin of *P. loescheii* has been purified and subjected to peptide analysis. An internal peptide, 10 amino acids in length, has been isolated from the digest and sequenced. A probe prepared by reverse translation has been used to identify a single cDNA restriction fragment that contains the adhesin gene. The DNA fragment is currently being ligated into a pBluescript construct for sequencing purposes. High molecular weight fibrillar structures (>2,000 kDa) from *P. loescheii* were isolated and purified. N-terminal amino acid analysis yielded the first 20 residues of the protein and a DNA probe was obtained by reverse translation. The probe identified six different clones in a lambda GT-11 phage library. A 3.8 kbp EcoRI digestion fragment was cloned into pBS KS(+) and the gene is currently being sequenced. One clone, pF428 synthesizes significant amounts of this protein.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00512-05 LME

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic Analysis of *Bordetella* and *Fusobacterium* Pathogenicity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Gentry-Weeks, Claudia, R., Senior Staff Fellow, LME, NIDR

Others: Thompson, John, Visiting Scientist, LME, NIDR

Diglisic, Gordana, Visiting Fellow, LME, NIDR

Spokes, Jennifer, Biologist, LME, NIDR

Robrish, Stanley, Research Microbiologist, LME, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Bacterial Toxins and Vaccines Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

2.83

PROFESSIONAL:

2.0

OTHER:

0.83

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Beta-cystathionase of *Bordetella avium* is a 42 kDa, homodimeric protein which is toxic for MC3T3-E1 osteogenic cells. Beta-cystathionase catalyzes the cleavage of L-cystine to pyruvate, ammonia, and thiocysteine. Transfer of sulfur from thiocysteine to MC3T3-E1 osteogenic cells results in inactivation of essential cellular enzymes and, ultimately, cell death. The goal of this study was to identify the amino acid residues of beta-cystathionase essential for catalytic activity. Genetic and physiochemical studies suggested that lysine residue 214 (K214) and cysteine residue(s) 88, 117, 279, and 309 are involved in enzyme activity. Therefore, these amino acids were replaced by alanine or glycine by site-directed mutagenesis of the beta-cystathionase (*metC*) gene. Mutant proteins, designated K214A, C88A, C117G, C279G, and C309A, were purified and assayed for specific activity, pyridoxal 5'-phosphate (PALP) co-factor, and binding of <sup>35</sup>S upon cleavage of L-<sup>35</sup>S-cystine. Mutant protein K214A had 5% of wild-type enzyme activity and 10% of K214A subunits contained the PALP co-factor, indicating that lysine residue 214 is the primary amino acid which binds co-factor but other residues and/or conformational properties contribute to PALP binding. Mutant proteins C88A and C279G retained 100% of wild-type activity and PALP binding. Replacement of C117 and C309 with alanine or glycine abolished beta-cystathionase activity and ability to bind PALP, indicating that these two residues are involved in PALP binding and thus, catalytic activity. All mutant proteins bound <sup>35</sup>S upon exposure to L-<sup>35</sup>S-cystine, suggesting that sulfur binding is non-specific and does not require catalytic activity. Since periodontal bacteria produce sulfur-containing compounds as byproducts of their metabolism, we examined *Fusobacterium* species for enzymes similar to beta-cystathionase. *F. nucleatum*, *F. periodonticum*, *F. necrophorum*, *F. necrogenes*, *F. mortiferum*, *F. russii*, and *F. varium* produced a protein which reacted with *B. avium* beta-cystathionase antibody. Extracts of *F. periodonticum* were toxic for MC3T3-E1 osteogenic cells and had beta-cystathionase activity. Cells treated with *F. periodonticum* extracts displayed striking resemblance to cells treated with *B. avium* beta-cystathionase. We hypothesize that beta-cystathionase of *F. periodonticum* contributes to periodontal disease by damaging osteogenic cells.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00514-05 LME

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Anthrax Toxin - A Model for Bacterial Pathogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Leppla, Stephen H., Research Chemist, LME, NIDR

Others: Klimpel, Kurt R., Staff Fellow, LME, NIDR  
Arora, Naveen, Visiting Associate, LME, NIDR  
Gordon, Valery A., Staff Fellow, LME, NIDR  
Stepanov, Alexey S., Visiting Fellow, LME, NIDR  
Haley, Sheila, A., Microbiologist, LME, NIDR

COOPERATING UNITS (if any)

Laboratory of X-ray Crystallography, Dana-Farber Cancer Institute (R.C. Liddington)  
Institute of Animal Health, Tsukuba, Japan (I. Uchida)  
Laboratory of Molecular Biology, NCI, NIH (D.J. FitzGerald)

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Bacterial Toxins and Vaccines Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

3.2

PROFESSIONAL:

2.37

OTHER:

0.83

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The structure and function of bacterial protein toxins is studied to determine how toxins contribute to bacterial pathogenesis. Mammalian cells are studied to identify the subcellular targets of the toxins.

A. Rapid progress occurred in a collaborative project to determine the structure of anthrax toxin protective antigen (PA) by X-ray diffraction. Mutant PA proteins containing single cysteine residues yielded heavy metal derivatives that helped to solve the structure. Approximately half the amino acids have been located in the three-dimensional structure.

B. PA residues 313-315, Phe-Phe-Asp, were shown by mutagenesis to be involved in translocation of the other two toxin components. Deletion of the two Phe residues prevented formation of a functional translocation channel. A mutant PA protein altered at residues 313-315 and at residues 164-167 was non-toxic and highly stable in bacterial culture supernatants. This protein may be useful in vaccines.

C. Additional evidence was obtained implicating the mammalian cell protease furin in the obligatory activation of bacterial toxins, including diphtheria toxin and anthrax PA. However, cell mutants lacking furin retained sensitivity to mutated toxins having sequences with paired basic amino acids that are not cleaved by furin, showing that cells contain at least one protease in addition to furin that activates toxins. Transfection with DNA vectors encoding furin confirmed that furin is the protease normally responsible for activating certain toxins.

D. Fusion proteins were produced that have residues 1-254 of anthrax toxin lethal factor (LF<sup>1-254</sup>) attached to catalytic domains from several other toxins. When combined with PA, these fusion proteins are highly toxic to mammalian cells because they are efficiently translocated to the cytosol. An LF<sup>1-254</sup> fusion to tetanus toxin light chain inhibited the growth of mouse macrophages and hamster cells, but not of several other cell types. This toxicity is attributed to cleavage of cellubrevin, a recently identified homologue of synaptobrevin, the target of tetanus toxin in neuronal cells. This fusion protein will help to define the role of cellubrevin, which is found in many different types of cells.

E. The previously identified activator of anthrax toxin gene transcription, the AtxA protein, was shown to bind to DNA regions upstream of *pag*, the PA structural gene. DNA from regions upstream of the LF and edema factor (EF) genes blocked AtxA binding to the *pag* promoter, evidence that AtxA regulates transcription of all three toxin genes. Deletion analysis of the *pag* promoter region showed that the AtxA protein binds to a site approximately 100 base pairs upstream of the transcriptional start site.



PROJECT NUMBER: Z01 DE00514-05 LME

PERIOD COVERED: October 1, 1993 to September 30, 1994

TITLE OF PROJECT: Anthrax Toxin - A Model for Bacterial Pathogenesis

PRINCIPAL INVESTIGATOR (AND OTHER PERSONNEL)

Keith, Jerry M., Chief, LME, NIDR

Singh, Yogendra, Guest Researcher, LME, NIDR

COOPERATING UNITS:

Department of Microbiology and Molecular Genetics, Harvard Medical School (R.J. Collier)

Division of Bacterial Products, CBER, FDA (J. Halpern)

Howard Hughes Medical Institute, Ann Arbor, Michigan (R. Kaufman, A. Rehemtulla)

Laboratory of Developmental Neurobiology, NICCHD, NIH (Y.P. Loh)





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00518-05 LME

PERIOD COVERED

October 01, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Detoxified Pertussis Toxin for Acellular Whooping Cough Vaccines

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Keith, Jerry M. Chief, Laboratory of Microbial Ecology LME, NIDR

Others: Merkel, Todd

Staff Fellow

LME, NIDR

COOPERATING UNITS (if any)

NIH, Tokyo Japan (H. Sato); Washington University, St. Louis, MO (R. Curtiss III); University of Missouri, Columbia MO (C. Parker)

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Bacterial Toxins and Vaccines Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.1

PROFESSIONAL:

1.1

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Whooping cough is caused by an infection of the respiratory tract with *Bordetella pertussis* bacteria. This disease is effectively controlled by the current vaccine which consists of killed whole *B. pertussis* cells. Though efficacious, the present vaccine produces unacceptable side effects. The major protective antigen in whooping cough vaccines is pertussis toxin. Clinical trials of acellular pertussis products strongly indicate that pertussis toxin will be a necessary and perhaps sufficient component of any new vaccine. Chemically "inactivated" pertussis toxin vaccines have been produced with reduced side effects and reasonable efficacy, however, residual activity may exist. Using site-specific DNA mutagenesis, we modified an *E. coli* subclone of the pertussis toxin SI subunit and then used these constructs to replace the chromosomal copy of the toxin gene in *B. pertussis* strain 3779. The resulting new strain produces a fully genetically detoxified form of pertussis toxin which is strongly immunoprotective and can be used as a vaccine antigen without chemical inactivation. Molecular studies are currently underway in our laboratory to develop high yield *B. pertussis* strains to enhance expression of pertussis toxin for use in acellular and conjugate vaccine manufacture.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00557-03 LME

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Specific Interactions of Bacteria with Mammalian Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Sandberg, Ann L. Chief, Microbial Recept. & Pathogen. Sect. LME, NIDR

Others: Sutphin, Michelle, Biologist, LME, NIDR

Ruhl, Stefan, Visiting Associate, LME, NIDR

Cisar, John O., Research Microbiologist, LME, NIDR

Takahashi, Yukihiro, Visiting Fellow, LME, NIDR

Yoon Jeong-Weon, Special Volunteer, LME, NIDR

Bryant, Joe, Chief, ACU, LME, NIDR

COOPERATING UNITS (if any)

Dr. Mike Eckhaus, VRP, NCRP, NIH; Jennie Owens, VRP, NCRP, NIH

Dr. Howard Jenkinson, Univ. Otago, Dunedin, New Zealand

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Microbial Receptors and Pathogenesis Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

4.08

PROFESSIONAL:

3.08

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☒ (b) Human tissues

☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Leukosialin (CD43) has been identified as the major polymorphonuclear leukocyte (PMN) receptor for both the Gal/GalNAc and sialic acid reactive lectins of *A. naeslundii* 12104 and *S. gordonii* Challis, respectively. This receptor was detected by bacterial overlays as well as Western and plant lectin blots of PMN extracts separated by SDS-PAGE. The streptococci bound directly whereas sialidase treatment of the transfers was required to unmask receptors for the actinomyces lectin. Unequivocal evidence of the receptor activity of CD43 was achieved by immunoprecipitation. Concentration by this latter technique also revealed a second putative receptor, CD45. Bacterial binding was inhibited by the appropriate saccharides and mutants or strains lacking lectins failed to adhere. The biological consequences of the recognition of the sialo- and asialoglycoconjugate receptors by the Challis and 12104 lectins, respectively, included stimulation of superoxide anion production, release of the contents of secondary PMN granules and phagocytosis. However, only the actinomyces were subsequently killed. Of six additional *S. gordonii* strains that also exhibited sialic acid lectin-dependent interactions with PMNs only two were killed although all were phagocytosed. The strains that resisted lectin-dependent killing by PMNs initiated severe endocarditis in a rat model whereas the two strains that were susceptible to killing induced only mild cardiac infection. The initiation of endocarditis failed to correlate with several previously described virulence determinants. Thus, resistance to lectin-mediated killing by PMNs appears to be of major importance for the induction of endocarditis by these strains. A mutant of Challis that had lost one major antigenic structure as well as sialic acid reactive lectin activity has been isolated. This mutant, however, retains other lectin activities recently detected with the parent and other *S. gordonii* strains. Studies have been initiated to assess the biological significance of these latter activities and their association with native bacterial structures.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00564-03 LME

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetics and Toxic Mechanism of Leukotoxins from Pathogenic Oral Bacteria

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Bouma, Carolyn L., Staff Fellow, LME, NIDR

Others: Holmes, Elisabeth, Biological Aide, LME, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Bacterial Toxins and Vaccines Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.5

PROFESSIONAL:

0.2

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Pathogenic oral bacteria are often associated with the progression of oral diseases, such as periodontitis. The Gram-negative bacterium *Actinobacillus actinomycetemcomitans* (Aa) colonizes periodontal sites and produces a leukotoxin that is likely to play an important role in periodontitis. Understanding the role of the Aa leukotoxin and other virulence factors in oral disease requires knowledge of the conditions under which such factors are produced, and how they act. Because of its probable role in periodontal disease, we have initiated studies of the genetics and mechanism of cytotoxicity of the Aa leukotoxin, LktA. *LktCA* has been isolated from Aa ATCC 29524 by PCR amplification, and we have developed fluorescent and isotopic assays for cytotoxicity. We have shown that cytoplasmic extracts of recombinant *E. coli* carrying *Aa lktCA* contain a protein that is cytotoxic to a human pre-monocyte cell line. The protein has been partially purified by S-sepharose chromatography, and is stabilized in the presence of denaturing agents (4M urea, 1% CHAPS). We have used recombinant DNA techniques to generate a histidine-labeled protein, which has been purified by Ni-NTA chromatography and will be used to prepare an anti-LktA antibody. The sites of transcription initiation for *Aa lktCA* have been identified. We have identified  $Ca^{2+}$  as an extracellular factor that modulates activity of the *lkt* promoter in Aa strain JP2. We propose to use Northern hybridizations to investigate other environmental factors that might affect LktA synthesis (temperature,  $CO_2$ , and others). We plan to use DNA hybridization as a tool to identify other oral bacteria that might produce leukotoxins. Genomic DNA from normal oral flora as well as organisms frequently isolated from diseased individuals will be included in this survey.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00571-02 LME

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Genetic Competence in Oral Streptococci

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Lunsford, R. Dwayne, Staff Fellow, LME, NIDR

Others: London, Jack P., Research Microbiologist, LME, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Clinical Microbiology

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The goal of this project is to study induction of genetic competence (competent for genetic transformation) in *Streptococcus gordonii* as a model system for global genetic regulation in the oral streptococci. Standard molecular genetic techniques are being utilized to isolate genes specifically induced at competence and to characterize the gene products responsible for DNA uptake and processing. This study will provide a set of developmentally induced loci with which to begin a systematic investigation of specialized gene expression in this genus. A second major aim of the project is to determine what role genetic transformation may play in the horizontal transfer of genetic information within the streptococcal cluster of the oral microbiota. These studies will determine whether competence has any relevance to antigenic variation and the overall genetic fitness for oral colonization by this group of organisms. Accomplishments during the covered period were:

- 1) Isolation of a cell wall/membrane DNA receptor
- 2) Isolation of a single-stranded DNA binding protein
- 3) Isolation of a putative competence factor gene
- 4) Development of a Tn4001 delivery system for oral streptococci.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00601-02 LME

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

HIV-Targeted Cytotoxic Proteins Derived from Anthrax Toxin

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Leppa, Stephen H., Research Chemist, LME, NIDR

Others: Klimpel, Kurt, R., Staff Fellow, LME, NIDR  
Gu, Mi-Li, IRTA Fellow, LME, NIDR;  
Teixeira, Avelino, Visiting Fellow, LME, NIDR  
Arora, Naveen, Visiting Associate, LME, NIDR  
Gordon, Valery M., Staff Fellow, LME, NIDR;

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Bacterial Toxins and Vaccines Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

4.65

PROFESSIONAL:

3.9

OTHER:

0.75

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Unique features of anthrax toxin are being exploited to make novel, cell-specific cytotoxins for HIV-1 infected cells. The three separate strategies described below for killing cells utilize highly-toxic fusion proteins in which the amino-terminal portion of anthrax toxin lethal factor (LF) is genetically fused to the ADP-ribosylation domain of Pseudomonas exotoxin A (PE). Delivery of these LF-PE fusion proteins to the cytosol of cells requires the prior binding and proteolytic activation of the protective antigen (PA) component of the toxin. Three approaches to targeting cells are being used:

1. The site in PA which must be proteolytically cleaved was replaced by consensus sequences recognized by HIV-1 protease, to make a mutant PA that will be activated only in HIV-1-infected cells. Four of five mutant PA proteins produced were cleaved by HIV-1 protease in vitro. Surprisingly, several of these were toxic with LF for normal, non-infected cells, showing that endogenous proteases were cleaving within the newly added sequences. It will be necessary to survey a larger number of target sequences to find ones cleaved by the HIV-1 protease but not by the endogenous cellular proteases.
2. CD4 and IL-2 were fused through a polypeptide linker to the carboxyl terminus of PA or PA deleted by 12 amino acids. The fusion proteins made from full size PA were more easily purified than those deleted by 12 amino acids. The fusion proteins retained some ability to bind to the PA receptor. Tests for the ability to bind to HIV-1 infected cells are in progress.
3. The gene encoding PA was transfected into several different mammalian cells to test the feasibility of sensitizing cells to LF fusion proteins by intracellular production of PA. No production of PA was obtained in initial tests.
4. A role for furin in processing HIV-1 gp160 was demonstrated using hamster cells that were either normal, furin-deficient, or expressing furin from a cDNA vector. Viral replication as tested by co-cultivation with T cells was greatest in the cells producing furin, showing directly that furin plays an essential role in viral replication.



Z01 DE00601-02 LME

Professional Personnel, continued

Chi, Angela  
Keith, Jerry M.

Biologist  
Chief

LME, NIDR  
LME, NIDR





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00604-01 LME

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetics and Biochemistry of Pentitol Metabolism in Oral Lactic Acid Bacteria

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Bouma, Carolyn, Staff Fellow, LME, NIDR

Others: London, Jack, Section Chief, LME, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Bacterial Toxins and Vaccines Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.85

PROFESSIONAL:

0.85

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

*Lactobacillus casei*, an oral bacterium prevalent in carious lesions, is one of only two lactic acid species capable of utilizing the pentitols xylitol (Xtl) and ribitol (Rtl) as growth substrates. Such polyols occur naturally in plants and are used as artificial sweeteners in sugar-free products. While cariogenic streptococci cannot metabolize these polyols, *L. casei* and *Enterococcus avium* are capable of producing acid from them. *L. casei* transports Rtl and Xtl via a specific phosphotransferase system (PTS). The Rtl and Xtl pathways each comprise a membrane-bound permease, a soluble III<sup>Rtl</sup> or III<sup>Xtl</sup>, and a pentitol-5-phosphate dehydrogenase. It is our goal to characterize the structure and function of the protein components of the Rtl and Xtl PTS pathways, and to study the genetic mechanisms underlying regulation of these systems. Our recent approaches involve the enzyme Rtl-5-P dehydrogenase. *RtlA* was cloned from an *L. casei* DNA library by hybridization and immunological screening. Plasmid subclones of *rtlA* directed the synthesis of a 49.5 kDa immunoreactive, enzymatically active protein in *Escherichia coli*. Nucleotide sequencing of these clones revealed a reading frame whose deduced amino acid sequence matched the amino-terminal sequence of purified Rtl-5-P dehydrogenase. A sequence motif found in NADH-dependent enzymes, GXGXXG, was found in the dehydrogenase sequence and is presumably the NADH binding site. The *RtlA* sequence shares identity with several NADH-dependent dehydrogenases, but is not related to the functionally similar dehydrogenases of the hexitol PTS of enteric bacteria. Another gene, designated *rtlR*, was identified as encoding a repressor protein. The deduced amino acid sequence of *RtlR* shares identity with the DeoR family of transcriptional repressors, including regulators of other PTS operons such as the *Streptococcus mutans* and *Lactococcus lactis* lactose PTS. It is presumed that the genes encoding the Rtl permease and III<sup>Rtl</sup> will be identified within the cloned *L. casei* DNA fragment. We propose to investigate the regulation of the *rtl* operon and to explore the relatedness of the *E. avium* and *L. casei* Rtl and Xtl PTS by immunological screening.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00607-01 LME

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Antibiotic Resistance among Streptococci

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Donkersloot, Jacob A., Research Microbiologist, LME, NIDR

Others: Pikis, Andreas, Staff Fellow, LME, NIDR

Harr, Robert J., Biolaboratory Technician, LME, NIDR

COOPERATING UNITS (if any)

William J. Rodriguez, Dept. of Infectious Diseases, Children's National Medical Center,  
Washington, D.C. 20010

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Bacterial Toxins and Vaccines Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.5

PROFESSIONAL:

1.1

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

*Streptococcus pneumoniae* is a significant cause of morbidity and mortality in pediatric, geriatric, and immunocompromised populations. Until the mid sixties, this pathogen was uniformly susceptible to penicillin, but during the last fifteen years the incidence of resistance to penicillin (as well as other antibiotics) has increased to such an extent around the world that infections due to penicillin-resistant pneumococci now pose a major threat in many countries. With regard to the USA, a nationwide survey showed that 5% of the pneumococci isolated in hospitals during the period 1979-'87 were intermediately resistant to penicillin (minimum inhibitory concentration (MIC) = 0.1-1 mg/L) and 0.02% highly resistant (MIC > 1 mg/L). As part of a Children's Hospital (Washington, DC) - NIDR program to provide research training for physicians, a pilot project was initiated to assess the prevalence of penicillin resistance among pneumococci isolated from normally sterile body sites of patients at Children's Hospital. The one-year survey (from June 1992 through May 1993) showed that 8.3% of the 108 strains isolated were intermediately resistant to penicillin and 4.6% were highly resistant. All isolates were susceptible to rifampin and vancomycin. However, at least 40% of the penicillin-resistant strains were also resistant to frequently used oral and parenteral cephalosporins (cefactor, cefixime, cefotaxime, cefpodoxime, cefuroxime, cephalixin, and loracarbef) and carbapenems (imipenem and meropenem). Also of great concern was the finding that all isolates tested were resistant to trimethoprim/sulfamethoxazole, a drug that is commonly used to treat infections in ambulatory patients. In addition (and, to our knowledge, for the first time), a penicillin-resistant strain with very high resistance to imipenem (MIC = 8 mg/L) was identified.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 DE00612-01 LME

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Stimulation of Cellular Immunity with Anthrax Lethal Toxin-Antigen Fusions

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Klimpel, Kurt, Staff Fellow, LME, NIDR

Others: Arora, Naveen, Visiting Fellow, LME, NIDR  
Leppla, Stephen, Research Chemist, LME, NIDR  
Keith, Jerry, M., Chief, LME, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Bacterial Toxins and Vaccines Section

INSTITUTE AND LOCATION

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TOTAL STAFF YEARS:

0.13

PROFESSIONAL:

0.13

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

*Bacillus anthracis* secretes two toxins into the extracellular medium during growth. The two toxins consist of three distinct proteins which combine in a pairwise fashion. Protective antigen (PA) can combine with lethal factor (LF) or edema factor (EF). PA combined with LF make lethal toxin while PA combined with EF make edema toxin. Part of each toxin is directly transported to the cytosol of living cells where it exerts its effect (see Arora, N., K.R. Klimpel, Y.Singh, and S.H. Leppla, J. Biol. Chem. 1992;267:15542-15548). We propose to take advantage of the efficient delivery of proteins to the cytosol to intracellularly inoculate living cells. Nearly all cell types have the ability to process proteins found in the cytosol and combine them with MHC class I molecules. The combination of the processed protein with the MHC class I molecule presented on the surface of the living cell results in the stimulation of a population of T-cells which recognize the processed protein antigen. Once stimulated, this population of T-cells can expand and become primed to rapidly respond to and eliminate cells which bear an identical combination of MHC class I and processed antigen. By making fusions between LF and different polypeptide antigens we may be able to vaccinate a host against many pathogens. Our initial work will focus on priming a response against several known antigenic proteins expressed by HIV-1, including p24, Nef, Env, Tat and Rev.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00423-09 LOM

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cloning, Expression and Characterization of Human Pancreatic Genes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Lan, Michael S.	Senior Staff Fellow	LOM, NIDR
Others:	DeSilva, Mark G.	Visiting Associate	LOM, NIDR
	Lu, Jia	Visiting Fellow	LOM, NIDR
	Li, Qing	Visiting Fellow	LOM, NIDR
	Donadel, Giulia	Visiting Associate	LOM, NIDR
	VanderVegt, F. Pierre	IRTA Fellow	LOM, NIDR
	Xie, Hong	Visiting Fellow	LOM, NIDR
	Notkins, Abner L.	Medical Director	LOM, NIDR

COOPERATING UNITS (if any)

BCDP-Dyn Corp., Program Resources, Inc.; Frederick Cancer Research and Development Center; Department of Pathology and Laboratory of Medicine, College of Medicine, University of Florida, Gainesville, FL.

LAB/BRANCH

Laboratory of Oral Medicine

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

7.12

PROFESSIONAL:

6.87

OTHER:

.25

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In this project, we used differential subtraction techniques to isolate genes from pancreatic beta cells. A human insulinoma cDNA library (ISL-153) was constructed by subtracting glucagonoma phagmid cDNA from insulinoma phagemid cDNA. A total of 153 clones was screened with different mRNA probes from insulinomas, glucagonoma and Hela cells. Clones that hybridized preferentially with end-labeled insulinoma mRNA were further screened by Northern analysis with a panel of tumor cell lines and normal tissues. cDNA sequences of selected clones were matched to the GenBank DNA database. Several novel cDNAs (i.e., IA-1, IA-2 and Pa-PDI) were further studied. Over the last year we localized IA-1 to chromosome 20p11.2, characterized its 5'-upstream regulatory elements and showed that it could serve as a neuroendocrine marker for human small cell lung carcinoma. IA-2 is a receptor-type protein tyrosine phosphatase. Northern blot analysis showed that IA-2 mRNA was expressed in five of five freshly isolated human insulinomas, rat and mouse insulinoma cell lines, and enriched normal mouse islets. It also was found in normal human brain, pituitary, pancreas, and brain tumor cell lines, but not in a variety of other normal or tumor tissues. Preliminary experiments indicate that a high percentage of patients with insulin-dependent diabetes mellitus make antibody to IA-2. Pa-PDI is a pancreas specific protein disulfide isomerase. It consists of two thioredoxin-like active sites (WCGHCQ, WCTHCK) and an ER retention signal sequence (KEEL) at the carboxyl terminus. Pa-PDI expressed in bacteria showed PDI enzyme activity. It catalyzed the reductive cleavage of radiolabeled insulin and renatured denatured RNaseA. Immunostaining of human tissues with a rabbit polyclonal antiserum raised against Pa-PDI indicated that this novel PA-PDI is expressed specifically in the acinar cells of the human pancreas. Other human pancreatic genes are currently being characterized.





Z01 DE00423-09 LOM

Professional Personnel, continued

Mange, Eloise

Editorial Assistant

LOM, NIDR



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 DE00471-07 LOM

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Transgenic Mice as Models for the Study of HIV-1 Pathogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Kopp, Jeffrey B.	Senior Research Investigator	LOM, NIDR
Others:	Notkins, Abner L.	Medical Director	LOM, NIDR
	Klotman, Paul E.	Special Expert	LOM, NIDR
	Kajiyama, Wataru	Visiting Associate	LOM, NIDR
	Marinos, Nancy J.	Biological Lab. Tech.	LOM, NIDR
	Wohlenberg, Charles R.	Microbiologist	LOM, NIDR
	Bryant, Joseph L.	Veterinarian	ACU, NIDR
	Davis, Harry	Animal Facilitator	ACU, NIDR

COOPERATING UNITS (if any)

M. Sporn, E. Bonninger, LCP, NCI; S. Thorgeirsson, LEC, NCI; C.C. Chan, NEI; G. Tudor-Williams, S. Sei, D. Kleiner, NCI

LAB/BRANCH

Laboratory of Oral Medicine

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

4.93

PROFESSIONAL:

3.43

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects    ☒ (b) Human tissues    ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Transgenic mice provide a unique model system to investigate molecular and cellular mechanisms of HIV-1 pathogenesis. Studies in HIV-transgenic mice from several laboratories, including our own, have helped to clarify the role of HIV-1 gene products in inducing disease independent of opportunistic infection. In previous work carried out in the LOM, a genetically-engineered HIV-1 proviral genome containing the *env*, *tat*, *rev*, *nef*, *vpr*, *vif* and *vpu* genes under the control of the HIV-1 long terminal repeat (LTR) promoter was introduced into the mouse genome by pronuclear microinjection. Transgenic mice expressing this viral transgene manifest several disease phenotypes that resemble some of the AIDS-related syndromes seen in HIV-infected patients. These include phenotypes similar to hyper-proliferative epidermal disorders, HIV-associated nephropathy, pediatric growth failure and wasting. These results implied a role for one or more of the HIV-1 gene products encoded by the viral transgene in the etiology of these pathologies. In the past year, we have analyzed new transgenic mice, which contain the HIV-1 LTR, and the viral proteins Env, Tat, Rev and Vpu. The level of HIV-1 gene expression in these transgenic mice was very low when compared with the level of expression observed in the former transgenic mouse line. Phenotypic abnormalities have included epidermal hyperproliferation and branching dysmorphogenesis affecting the salivary gland, mammary gland, liver, and kidney. These findings expand the pathogenic role of HIV-1 proteins in transgenic mice, and suggest complex interactions with host proteins.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00534-04 LOM

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Human Immunoglobulin Genes and their Properties

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Cheung, Sau C.	Staff Fellow	LOM, NIDR
Others:	Donadel, Giulia	Visiting Associate	LOM, NIDR
	Kajiyama, Wataru	Visiting Associate	LOM, NIDR
	Notkins, Abner L.	Medical Director	LOM, NIDR
	Mange, Eloise	Editorial Assistant	LOM, NIDR

COOPERATING UNITS (if any)

Dr. E. Padlan, NIDDK

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Laboratory of Oral Medicine

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INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.45

PROFESSIONAL:

1.2

OTHER:

.25

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects    ☐ (b) Human tissues    ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Polyreactive antibodies recognize many structurally dissimilar self and foreign antigens. The mechanism by which polyreactive antibody interacts with antigens has not been elucidated. The elucidation of the molecular basis of polyreactivity would be of great value to the understanding of molecular recognition. The objectives of this project are to determine the precise antigen-binding site and to dissect the molecular properties underlying the mechanism of polyreactivity. A human polyreactive monoclonal IgM antibody (mAb 67) has been selected for detailed structural analysis. The genes encoding the Fd heavy and light chains were cloned and the Fab fragment and single-chain Fv (scFv) gene constructs were generated and expressed in E. coli. The results showed that biologically active recombinant Fab and scFv fragments were produced in E. coli. ELISA and Western blot analyses showed that these recombinant antibody molecules bound to many self and nonself antigens. These results demonstrate that the antigen-binding activity of polyreactive antibodies resides in the hypervariable regions and thus eliminate speculation that Fc domain or carbohydrate moiety contribute to polyreactivity. It is possible, however, that the antibody-combining site might be partitioned into different interacting areas. To explore this possibility, we generated a hybrid antibody construct, in which the genes encoding the CDR-3 of heavy and light chains from mAb 67 were transplanted by over-lapping extension PCR onto a structurally compatible non-polyreactive antibody from which the corresponding CDR genes were removed. This construct will be expressed in bacteria to determine whether a specific area of the CDR possesses polyreactivity.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 DE00536-04 LOM

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Polyreactive Antibodies and Gene Deletion

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Sigounas, George	Visiting Associate	LOM, NIDR
Others:	Notkins, Abner L.	Medical Director	LOM, NIDR
	Monell-Torrens, Esteban	Bio. Lab. Technician	LOM, NIDR
	Trado, Dorothy	Secretary	LOM, NIDR
	Mange, Eloise	Editorial Assistant	LOM, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Oral Medicine

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.6

PROFESSIONAL:

1.1

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Polyreactive antibodies are natural antibodies capable of binding multiple antigens. These antibodies are commonly found in healthy and diseased individuals. To determine the level of polyreactive antibodies in the blood and explore the biological properties of polyreactive antibodies we performed in vitro studies with human monoclonal and polyclonal antibodies obtained either from hybridoma cell lines or human plasma. We found that affinity-purified IgM showed many times more reactivity with a panel of antigens compared to human plasma containing the same amount of IgM. When plasma was added back to the affinity-purified IgM, the reactivity of the IgM with antigens was completely inhibited. When the affinity-purified IgM was affinity-purified a second time by passage through antigen-specific columns, the eluted antibodies bound not only to the antigen used for purification, but also to a panel of unrelated antigens, indicating that the antibodies were polyreactive. It is concluded that polyreactive IgM antibodies are present in the circulation, but are masked by binding to circulating antigens.

Several studies have shown that polyreactive antibodies are produced, at least in part, by CD5 antigen-bearing B cells. To understand the relationship between the expression of the CD5 gene and the production of polyreactive antibodies, we cloned, mapped and determined the exon-intron boundaries of the genomic form of the CD5 gene. We also introduced mutations into the second and third exons. The mutated genes have been introduced into embryonic stem (ES) cells by electroporation. Six clones with the recombined CD5 gene have been isolated. These mutated ES clones are now being introduced into blastocysts to generate chimeric mice.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00562-03 LOM

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Biology of HIV and Gene Therapy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Rappaport, Jay	Senior Staff Fellow	LOM, NIDR
	Klotman, Paul E.	Special Expert	LOM, NIDR
Others:	Bruggeman, Leslie	Senior Staff Fellow	LOM, NIDR
	Franks, Roberta	Senior Staff Fellow	LOM, NIDR
	Franko, Maryrose	Special Volunteer	LOM, NIDR
	Hanss, Basil	Special Volunteer	LOM, NIDR
	Richardson, Max	Biologist	LOM, NIDR
	Notkins, Abner L.	Medical Director	LOM, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Oral Medicine

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

6.3

PROFESSIONAL:

4.45

OTHER:

1.85

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Human Immunodeficiency Virus is the causative agent of AIDS. Since viral loads correlate with disease progression, strategies designed to inhibit HIV replication may be beneficial in the treatment of disease. We have developed different strategies to inhibit HIV replication. These strategies are useful in inhibiting virus replacation in culture and we are now testing the efficacy and toxicity of these strategies in a mouse HIV model. We have generated transgenic mice with ribozymes targeted to HIV message. Similarly, transgenic animals have been generated which contain a construct with multiple copies of the Tat activation response element TAR. Over-expressed TAR RNA may inhibit HIV expression either by competing for Tat or cellular factors involved in transactivation. We are mating these animals with transgenic animals containing a defective HIV provirus lacking the gag-pol region. These HIV transgenics exhibit some of the manifestations of AIDS-associated diseases including proliferative skin lesions and renal disease similar to HIV-associated nephropathy. Studies are in progress to determine the transcriptional and post-transcriptional mechanisms which regulate HIV gene expression in the transgenic mouse model. Transcription factor NF-kB as well as factors which regulate RNA splicing patterns are responsible for tissue-specific control of HIV regulation. In addition to these strategies, we are pursuing studies with phosphorothioate antisense oligonucleotides as a potential therapeutic. Our results in vivo suggest that antisense is actively taken up by renal cells and is not merely passively filtered. Studies are in progress to determine the identity of these putative transporters. As an additional approach to inhibiting HIV gene expression, we are exploring the interaction between HIV-1 and the weakly pathogenic HIV-2 cells which contain both viruses. Our results suggest that HIV-1 is inhibited by HIV-2. HIV-2 inhibits HIV-1 gene expression and the inhibitory activity can discriminate between HIV-1 and HIV-2 TAR elements.



Z01 DE00562-03 LOM

Professional Personnel, continued

Trado, Dorothy

Secretary

LOM, NIDR





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 DE00619-01 LOM

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Intracellular Immunization

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Zhou, Paul	Senior Staff Fellow	LOM, NIDR
Others:	Devadas, Krishna	Visiting Associate	LOM, NIDR
	Babbott, Cecelia	IRTA Fellow	LOM, NIDR
	Notkins, Abner L.	Medical Director	LOM, NIDR
	Trado, Dorothy	Secretary	LOM, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Oral Medicine

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.02

PROFESSIONAL:

1.77

OTHER:

.25

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

Antibodies work extracellularly. During the past several years, a great deal of information on cell compartmentalization has accumulated. Several anchor domains responsible for intracellular localization of a given protein to the endoplasmic reticulum (ER), trans-golgi network (TGN) or nucleus have been identified. As a result, it is becoming possible to target genetically engineered molecules to specific cell compartments by linking the molecules to be expressed with specific anchor domains. The purpose of the current project is to determine whether antibody genes can be directed and expressed in appropriate cell compartments and if so whether they will affect physiologic and pathologic processes.

The model system that is being developed involves the expression of anti-HIV antibody genes in human T cell lines. Heavy and light chain antibody genes specific for HIV-1 gp120, gp41, and protease have been isolated. Single chain Fv gene constructs specific for gp120 and gp41 linked with or without ER and TGN anchor domains have been generated, cloned into mammalian expression vectors and transfected into human T cell lines. In vitro assay demonstrated that these constructs were able to make a fair amount of the right size proteins. The expression, cell localization, and antigen-binding capacity of these transgenes are now being studied. If the transgenes are properly expressed, the transfectants will be used as targets for HIV infection and the inhibitory effect of the intracellular anti-HIV antibodies on the replication, assembly and egress of HIV will be assessed. If a significant inhibitory effect occurs, attempts will be made to introduce anti-HIV antibody genes into human hematopoietic stem cells from HIV infected individuals with the hope that when the stem cells mature into HIV-susceptible CD4 T cells the intracellular antibody will protect or at the least limit the degree of expression of HIV.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00620-01 LOM

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Antigen-Binding B Cells and Polyreactive Antibodies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Chen, George	Senior Staff Fellow	LOM, NIDR
Others:	Shimizu, Fumio	Guest Researcher	LOM, NIDR
	Qian, Jiahua	Special Volunteer	LOM, NIDR
	Notkins, A.L.	Medical Director	LOM, NIDR
	Wheeler, James	Biologist	LOM, NIDR
	Trado, Dorothy	Secretary	LOM, NIDR
	Mange, Eloise	Editorial Assistant	LOM, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Oral Medicine

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

3.07

PROFESSIONAL:

1.57

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Polyreactive antibodies (Abs) are naturally occurring Abs, primarily of the IgM, but also of the IgG and IgA isotypes. These Abs are capable of reacting with a variety of antigens (Ags) which may differ greatly one from another. The present experiments were initiated to see if B lymphocytes capable of binding Ags could make polyreactive Abs. Fluorescein isothiocyanate labeled self and non-self antigens were incubated with B lymphocytes from normal individuals. Ag binding lymphocytes were separated from non-Ag binding lymphocytes by the fluorescence-activated cell sorter (FACS), immortalized with EB virus and analyzed at the clonal level for their capacity to make polyreactive Abs. Four-to-six times more cells making polyreactive antibodies were found in the B lymphocytes subset that bound Ags than in the B lymphocytes subset that did not bind Ags. The majority of the polyreactive Abs were IgM. Immunoflow cytometry revealed that B cell lines making polyreactive Abs bound a variety of antigens (e.g. insulin, IgGFc and  $\beta$ -galactosidase) whereas B cell lines making monoreactive antibodies bound only a single Ag. The binding of Ags to B cell lines that made polyreactive Abs could be inhibited (28%-57%) by both homogeneous and heterogeneous Ags. Both CD5+ and CD-Ag binding B cells made polyreactive Abs, but the frequency was slightly higher in the CD5+ Ag binding (85%) as compared to the CD5- Ag binding (50%) population. Comparison of CD5+ B cells that bound Ags with CD5+ B cells that did not bind Ags showed that approximately 85% of the former, but only 15% of the latter, made polyreactive Abs. It is concluded that cells capable of making polyreactive Abs can bind a variety of different Ags and that Ag binding is a good marker for identifying polyreactive Ab producing cells.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00031-26 NAB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Design and Computer Interfacing of Neurophysiological Instrumentation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Brown, Frederick

Electronic Engineer (Instru)

NA NIDR

Others:

COOPERATING UNITS (if any)

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Nociception and Tissue Injury Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

These projects involve the design and construction of electronic and electromechanical instrumentation to be used in neurophysiological, physiological and behavioral research. Projects also include the interfacing of these and other instruments to laboratory computers. Electronic circuit design, microcomputers, and assembly language programming may be used in these instruments or interfaces.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00132-20 NAB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pharmacologic Modulation of Neuroendocrine Responses to Stress and Inflammation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Dionne, Raymond	Chief, Clinical Pharmacology Unit	NA NIDR
Others: Gordon, Sharon	NRSA Fellow	NA NIDR
Dubner, Ronald	Chief, NAB, IRP	NA NIDR

COOPERATING UNITS (if any)

McCullough, Linda	Staff Nurse	CC Nursing
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LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Nociception and Tissue Injury Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.4

PROFESSIONAL:

1.1

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

- |                                                        |                                            |                                      |
|--------------------------------------------------------|--------------------------------------------|--------------------------------------|
| <input checked="" type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors                   |                                            |                                      |
| <input type="checkbox"/> (a2) Interviews               |                                            |                                      |

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objectives of this project are 1) to evaluate the neuroendocrine responses to surgical stress and inflammation, 2) to determine the analgesic and anti-inflammatory effects of prototype and novel drugs which alter either the synthesis or the receptor activation of neuroendocrine mediators, and 3) to evaluate the clinical utility of these novel drugs in controlled clinical trials. Previous work on the physiologic function of plasma beta-endorphin and regulation of its release has provided evidence of enhanced release by a variety of stressors, including clinical pain following oral surgery and chest pain in patients with coronary artery disease. A current clinical study is attempting to measure inflammatory mediators released following surgery in order to determine the relationship between local levels of inflammatory mediators, clinical reports of pain, and their modulation by local infusion of prototypic drugs into the site of inflammation. Subjects undergoing the removal of an impacted third molar have a microdialysis fiber placed under the mucoperiosteal flap which is then slowly perfused to allow diffusion of mediators from the extracellular fluid into the perfusion fluid. A preliminary study demonstrated the ability to measure prostaglandin E2 in the inflammatory exudate which increased postoperatively with the onset of acute postoperative pain. Systemic administration of ketorolac decreased both pain and PGE2 levels, suggesting a functional relationship between NSAID analgesia and levels of locally released PGE2. A current study is evaluating the effects of very low doses of ketorolac (1 mg) administered directly into the extraction site at pain onset in comparison to systemic administration of the same dose. Demonstration of a locally-mediated effect at a dose too low to produce systemic effects will provide a basis for further studies to evaluate the effects of prototypic and novel drugs on other mediators of pain and inflammation. Increasing evidence suggests that the nociceptive afferent barrage which can occur during a surgical procedure can activate central processes leading to an increased perception of clinical pain long after the nociceptive input is removed. This hypothesis is being evaluated in the oral surgery model by randomly allocating local anesthesia or placebo anesthesia prior to the surgical removal of third molars with general anesthesia. Interim data analysis suggests that pretreatment with a long-acting local anesthetic reduces acute pain by 50% at the 48 hour observation, long after the local anesthetic has dissipated. If confirmed, these data provide evidence to support the hypothesis that nociceptive afferent barrage produces central plasticity leading to increased postoperative pain.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00133-20 NAB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Assessment of Experimental and Clinical Pain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Gracely, Richard H.	Research Psychologist	NA NIDR
Others: Dionne, Raymond	Research Pharmacologist	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR
Max, Mitchell B.	Chief, Clinical Trials Unit	NA NIDR
Smith, Wendy	Psychologist	NA NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neuropathic Pain Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.46

PROFESSIONAL:

0.7

OTHER:

0.76

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Five experiments were performed. Two focused on the recall of pain reports. Thirty-two cancer patients with chronic pain showed an unexpected contrast effect after pain was increased by physical therapy in one of two groups. Patients with increased pain recalled lower past pain and greater medication efficacy. Recall accuracy can influence diagnosis, choice of treatment and evaluation of treatment efficacy. The second recall study evaluated the influence of expected post surgical pain following extraction of third molar teeth. Actual postoperative pain was lower than expected pain, and pain recalled after 1 week was accurate and not influenced by the ratings of expected pain. The remaining experiments used pain evoked experimentally by a thermal probe in 3 different paradigms. In the third, the track-ball method (continuous manipulation of a visual display) was used to assess the effects of fentanyl or placebo on several components of continuous pain ratings of sensations evoked by 3-sec thermal stimuli of varying intensity. A multivariate analysis of variance showed that an evaluation using four components of the response wave form was more sensitive than analyses using only one component, such as the standard measure of pain intensity. Another experiment used track-ball assessment of trains of six 49°C stimuli to assess the effects of the substance P blocker, CP39,994-1, and fentanyl on pain mediated by A-delta and C-fiber nociceptors. Preliminary analysis showed that this method can discriminate between these pain components without any instruction (and potential bias) about the existence of these two sensations. The effect of the substance P blocker on these ratings will be evaluated once the code is broken. It is expected that the substance P blocker will attenuate the spinal summation of C-fiber mediated sensation demonstrated with this method, providing a useful model for clinical states of hyperexcitability. The fifth experiment used the interactive staircase method to evaluate pain sensitivity in patients with sickle cell disease and the efficacy of oral morphine on these sensations. This method previously showed that patients with "sensitive hearts", i.e. angina-like symptoms but no coronary artery disease, had decreased cutaneous pain sensitivity, refuting a hypothesis of generalized pain hyper-sensitivity. Preliminary results suggest that patients with sickle cell disease show an opioid tolerance characterized by an initial modest analgesic response at 2.5 hours and a subsequent hyperalgesic response 5 hours after administration of 30 mg of slow release morphine.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00286-15 NAB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Experimental Therapeutics for Acute Pain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Dionne, Raymond Chief, Clinical Pharmacology Unit NA NIDR

Others: Berthold, Charles Guest Scientist NA NIDR

COOPERATING UNITS (if any)

Rowan, Janet	Nurse	CC Nursing
Parada, Susan	Nurse	CC Nursing

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Nociception and Tissue Injury Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

0.8

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The project consists of a series of clinical trials evaluating the clinical efficacy and safety of experimental therapeutic agents for the control of acute pain and peri-operative apprehension in ambulatory patients undergoing minor surgical procedures. The surgical removal of impacted third molars serves as a model of minor surgery with associated intraoperative and postoperative pain and peri-operative apprehension. All studies are double-blind with randomly allocated, parallel treatment groups and multiple dependent measures of therapeutic efficacy and clinical safety. The efficacy of nonsteroidal anti-inflammatory drugs applied peripherally at the site of injury was evaluated in a series of three clinical trials. A proprietary formulation of ketoprofen was administered directly into third molar extraction sites in comparison to a placebo formulation. Administration of a dose which was 20% of the normal systemic dose suppressed postoperative pain over a six hour observation period in comparison to placebo. A second study demonstrated that comparison of administration at the extraction site to oral administration of the same dose of the gel formulation, with a placebo control, resulted in significantly less pain only following administration into the peripheral site. A recently completed study evaluated blood levels following administration of the same dose both peripherally and orally to rule out analgesic activity due to a systemic effect following absorption from the extraction site. The results of these studies supports the hypothesis being evaluated that peripheral administration of an NSAID at low doses results in greater analgesic efficacy and a lower incidence of side effects by minimizing systemic exposure. A parallel study is evaluating the analgesic efficacy of a receptor antagonist of substance P. The drug is administered prior to, during, and following the surgery in an attempt to block occupancy of the substance P receptor and possible CNS activation of nociceptive processes which can persist after removal of the painful peripheral input. {Preliminary results by next week -- see important note under Major Findings} A previous study demonstrated that oral administration of a benzodiazepine hypnotic agent produces anxiolytic activity in the oral surgery model comparable to parenteral administration of diazepam. A study completed this year demonstrated that sublingual administration of triazolam results in even greater efficacy than oral administration without any concomitant increase in psychomotor effects. These results hold promise for the clinical use of a single entity, non-parenteral form of sedation with efficacy comparable to parenteral administration but with greater safety.







DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00288-15 NAB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization of the Molecular Response to Noxious Stimulation and Nerve Injury

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Ruda, M.A.	Chief, CMM Section	NA NIDR
Others: Allen, Barbara	Biologist	NA NIDR
Franklin, Emma	Biological Lab. Tech.	NA NIDR
Ren, Ke	Visiting Associate	NA NIDR
Zhang, Rui-Xin	Visiting Scientist	NA NIDR
Chhatpar, Ravi	Summer Student	NA NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Cellular and Molecular Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

3.36

PROFESSIONAL:

1.3

OTHER:

2.06

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

These research projects extend our previous analysis of the neuronal response to noxious stimulation of the periphery and nerve injury. Neurons in the dorsal root ganglion and spinal cord represent the first level of processing of neuronal information from the periphery. Using cellular and molecular techniques it is possible to identify important elements in the neuronal networks that subserve the response to nociception, nerve injury and regeneration. The descending control of nociceptive neuronal circuits is being studied in animals with spinal cord transection and inflammation of one hindpaw. Following spinal transection, complete Freund's adjuvant (CFA) hindpaw injection produces edema that is comparable in magnitude in the sham operated control rats and those with spinal transection. RNA blot analysis of spinal cord peptide and transcription factor genes known to be regulated in our animal model of peripheral inflammation and hyperalgesia will provide new insight into the mechanisms of descending control of nociceptive neuronal circuits. Receptor sites on neuronal membranes represent the initial phase of neuronal activation. Recently, several of the genes that encode receptors important to processing of nociceptive inputs have been cloned. We have begun a systematic analysis of receptor mRNA expression in the spinal cord and dorsal root ganglia in response to noxious stimulation. The NMDA NR2C receptor which has a low level of constitutive mRNA expression in the spinal cord was not regulated at the level of its mRNA following noxious stimulation. Similarly, little change was seen on RNA blot analysis for the KA2, neurotensin and cannabinoid receptor mRNAs ipsilateral to the inflamed hindpaw. These preliminary observations suggest that receptor regulation may not generally occur at the level of mRNA expression in response to nociceptive stimulation.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DE 00329-13 NAB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Discrimination of Thermal Stimuli Applied to the Face in Monkey

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Kenshalo, Jr., Daniel R.	Research Biologist Neurobiology	NA NIDR
Others: Dubner, Ronald	Chief, NAB	NA NIDR
Douglass, Diana	Postdoctoral Fellow	NA NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Nociception and Tissue Injury Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

2.3

PROFESSIONAL:

2.1

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project investigates the neural mechanisms that subserve the monkey's ability to detect innocuous cutaneous stimuli (air puffs) delivered to the face. The magnitude of sensations produced by small increases in air puff stimuli was studied with use of a reaction time paradigm. The monkey initiated a trial by pressing an illuminated button. Subsequently air puff stimuli (AP1) of identical intensity were delivered to the face at the rate of one per second. After a variable time period between 4 and 10 seconds, an air puff of higher intensity (AP2) was presented. The subject was required to release the button as soon as the larger air puff stimulus was detected. Discrimination speed was defined as the reciprocal of the time interval between the onset of the larger air puff stimulus and the release of the button. The monkey's discrimination speeds to stimuli presented on the face were dependent on the intensity of the AP2. The psychophysical functions obtained from the monkey's face were monotonically related to the intensity of AP2. As the intensity of AP2 increased, the monkey's discrimination speeds shortened. Neuronal recordings were made from the medullary dorsal horn (MDH) as the monkey performed the psychophysical task. Responses produced by air puff stimuli were examined in low, threshold mechanosensitive (LTM) and wide-dynamic-range (WDR) neurons. Subpopulations of WDR and LTM encoded the intensity of innocuous, air puff stimulation. However, the slope of the stimulus-response functions to air puff stimuli was greater for WDR neurons. Therefore, WDR neurons exhibited greater sensitivity to air puff stimuli than LTM neurons. The relationship between discrimination speed and peak neuronal discharge was examined for WDR and LTM neurons. There was a higher correlation between the peak frequency of discharge and discrimination speed for WDR neurons as compared to LTM neurons. This evidence indicates that a subpopulation of WDR and LTM neurons may provide information for the monkey to discriminate innocuous, air puff stimuli delivered on the face. However, WDR neurons are capable of providing more information concerning the intensity of air puff stimulation and their discharge is more predictive of the monkey's discrimination ability.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00366-12 NAB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Analgesic Mechanisms in Patients with Chronic Pain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Max, Mitchell B.	Chief, Clinical Trials Unit	NA NIDR
Others: Park, Karen	IRTA Fellow	NA NIDR
Sethna, Navil	Clinical Associate	NA NIDR
Sang, Christine N.-M.	Clinical Associate	NA NIDR
Nelson, Kristine	IRTA Fellow	NA NIDR
Liu, Maywin	Special Volunteer	NA NIDR

COOPERATING UNITS (if any)

Robinovitz, Elaine	Nurse	CC Nursing
Parada, Sue	Nurse	CC Nursing

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neuropathic Pain and Pain Measurement Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

3.25

PROFESSIONAL:

3.05

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to elucidate the neural mechanisms and principles of treatment of chronic pain syndromes, with particular attention to the drug treatment of pain caused by nerve injury. Because of growing evidence from animal studies that neuropathic pain may be mediated by NMDA receptor-mediated activation of spinal cord neurons, NMDA receptor antagonists are being studied in chronic pain in patients with nerve damage and experimental pain in normal volunteers, consisting of hyperalgesia of the skin briefly induced by intradermal injection of capsaicin. Twenty-two of a projected 24 patients with painful diabetic neuropathy or postherpetic neuralgia have entered a placebo-controlled, double-blind, crossover comparison of high-dose oral extromethorphan, an NMDA receptor antagonist. Four of the 14 patients who have completed the initial study reported substantial pain relief on dextromethorphan, relative to placebo, and have entered a sequence of repeated drug-placebo comparisons to see if these responses are consistent. Previous studies showed that the NMDA receptor antagonist ketamine and the opioid agonist alfentanil reduced capsaicin-evoked pain and hyperalgesia in volunteers. Therapeutic effects only occurred at doses that also caused cognitive impairment, however. In order to examine whether combining the NMDA antagonist with the opioid enhances analgesia more than side effects, we compared two doses each of ketamine alone and alfentanil alone to a combination of the two drugs in normal volunteers, assessing pain, hyperalgesia, and cognitive side effects. Relative to each drug alone, the combination showed simple additive effects for pain relief and cognitive effects, suggesting that a systemically administered combination of these drugs might not provide a therapeutic advantage in patients whose chronic pain was produced by similar neural mechanisms i.e. sensitization of central pain-signalling neurons. In order to improve the therapeutic ratio of NMDA receptor antagonists, we are pursuing clinical trials with other classes of these drugs, and once data is available from an NIDR contract for neuropathology studies of spinally administered NMDA receptor antagonists, we will pursue studies of the spinal route of administration, which has been effective in animals and minimizes the amount of drug reaching the brain.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00413-09 NAB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Experimental Neuropathy of Peripheral Nerve in Rat

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Bennett, Gary J.	Chief, NPPM Section	NA NIDR
Others: Tal, Michael	Visiting Associate	NA NIDR
Xiao, Wen-Hau	Visiting Fellow	NA NIDR
Imamura, Yoshiki	Special Volunteer	NA NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neuropathic Pain and Pain Measurement Section

INSTITUTE AND LOCATION

NIH, NIDR, Bethesda, MD 20892

TOTAL STAFF YEARS:

2.74

PROFESSIONAL:

2.14

OTHER:

0.6

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Rats with an experimental peripheral neuropathy to the sciatic nerve have behavioral symptoms that indicate disordered pain sensations like those seen in humans with nerve damage due to trauma and disease. In particular, the rats have hyperalgesia to thermal and mechanical stimuli, allodynia (pain from normally innocuous stimuli) to touch, and spontaneous pain (or dysesthesias). In humans, the abnormal pain sensations are sometimes found in areas that do not correspond to the individual or aggregate territories of peripheral nerves or dermatomes. The similarity of the spatial distribution of such pain to the "anatomically impossible" distribution of hysterical paralysis has led some physicians to conclude that the patients have a neuroses rather than an organic neurological disorder. We have shown that the nerve-injured rats also have this extra-territorial pain and that the pain from outside the territory of the injured nerve is initiated by input carried over adjacent uninjured nerves. These results show that extra-territorial pain may have an organic basis: a disorder of sensory processing in the central nervous system. Work in the clinic and in the laboratory has led to the hypothesis that neuropathic pain has two fundamental causes, one at the level of the nerve injury, a source of ongoing nociceptor discharge, and one at the level of the spinal cord, a central disorder of sensory processing mediated by activity at glutaminergic synapses of the N-methyl-D-aspartate (NMDA) receptor type. Targeting the peripheral pathology, we have shown that the application of SNX-111 and SNX-124, synthetic versions of omega-conotoxins that are highly selective blockers of N-type voltage-sensitive calcium channels, blocks the rat's abnormal pain sensations, presumably by reducing calcium channel-mediated spontaneous discharge in injured nociceptors. Targeting the central pathology, we have shown that the NMDA receptor blockers/modulators, dextrophan, magnesium, and Felbamate, suppress some but not all of the rat's abnormal pain sensations. These results confirm the NMDA receptor hypothesis but suggest that other types of synapses are involved in the production of some of the symptoms.





**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 DE 00414-09 NAB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

**CNS Neurotransmitter Regulation During Peripheral Inflammatory States**

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Iadarola, Michael J.	Research Pharmacologist	NA NIDR
Others: Gu, Jun	Visiting Fellow	NA NIDR
Messersmith, Donna J.	IRTA Fellow	NA NIDR
Dubner, Ronald	Chief, NAB, NIDR	NA NIDR
Kim, David	Technician	NA NIDR
Lee, Susan	HHMI Summer Student	NA NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Nociception and Tissue Injury Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

4.4

PROFESSIONAL:

3.0

OTHER:

1.4

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project focuses on the transcriptional control of the prodynorphin gene, which codes for the dynorphin family of opioid peptides. During peripheral inflammation dynorphin gene expression is greatly increased in spinal cord neurons where the neuropeptide products can modulate chronic pain. Transient transfection data using in vitro cell lines now indicate that the dynorphin gene is turned on via activation of the cyclic AMP (cAMP) second messenger system. Transient co-transfections demonstrate that transactivation can be mediated by various proteins from distinct transcription factor families. A dual feed-forward stimulatory transcription scheme is suggested in which members of the CREB/ATF and Fos/Jun families positively transactivate prodynorphin gene expression. These results yield a new level of insight into gene regulatory processes induced by persistent pain states. The results imply that a transmitter in the primary afferent neurons is linked to stimulation of adenylate cyclase in second order neurons and that some of the neuronal hyperexcitability changes that accompany chronic pain may be engendered or maintained by cAMP-dependent phosphorylation events. The cAMP responsive element is located far upstream (-1546 bp) from the transcription start site. This sequence, which is not an exact cAMP response element (CRE) consensus, is called the DYNCRE III element and can act in conjunction with two more CRE-like elements located 100 bp more 5 prime (DYNCRE I and DYNCRE II). We have attempted to block cAMP induction by co-transfection with vectors expressing inhibitory mutations of transcription factor proteins. The cAMP-induced increase can be attenuated by expression of a dominant negative mutant of the c-Jun protooncogene which can heterodimerize with Fos as well as CREB and both families (i.e. Fos/Jun and CREB/ATF) are capable of producing strong transactivation of the dynorphin gene upon co-transfection with vectors that express intact protein. Mutants of CREB are also being tested. Thus, regulated expression of the dynorphin gene in the spinal cord and other brain regions (e.g. striatum) may be dependent on the amount and the phasic or tonic nature of neurotransmitter stimuli coupled to cAMP. The upstream CRE sequences are probably not the sole elements controlling dynorphin gene expression. Previous studies from our group indicated one such element at -208 to -216 which we have termed upstream regulatory element (URE). We have cloned a protein, Ure binding protein 1 (UREB1), which specifically binds the URE. Western blotting with two antisera to this protein indicate a nuclear localization using the antiserum against the recombinantly expressed protein. However, the C-terminally-directed antiserum identifies immunoreactivity in the cytoplasmic compartment suggesting that UREB1 may undergo post-translational proteolytic processing prior to nuclear translocation. Protein sequence analysis has disclosed that UREB1 is homologous to three proteins of largely unknown function: a yeast open reading frame, a cDNA from mouse brain and a human protein termed E6-AP. These studies elucidate the pivotal role of the spinal dynorphin system in pain mechanisms and may provide new avenues for the pharmacotherapy of pain and insights into chronic opioid use and tolerance.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00440-08 NAB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neural Mechanisms of Experimental Hyperalgesia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Ren, Ke	Visiting Associate	NA NIDR
Others: Ruda, Maryann	Chief, CMM Section	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR
McCarthy, Shelly	Summer Intern	NA NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Nociception and Tissue Injury Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1

PROFESSIONAL:

0.7

OTHER:

.3

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

To search for new and improved therapies for pain, these experiments were undertaken to examine the mechanisms underlying the hyperalgesia following peripheral nerve or tissue injury. A new series of experiments is initiated to study the modulation of dorsal horn hyperexcitability by brain centrifugal systems. Fourteen nociceptive neurons were recorded from the superficial dorsal horn in complete Freund's adjuvant (CFA)-inflamed rats. Our previous studies have established that there is an increase in spinal neuronal activity in inflamed animals. A reversible spinal cord block was produced by application of 2% lidocaine onto the thoracic spinal cord. After the application of lidocaine, there was an increase in background activity, further expansion of the receptive fields, and an increase in responses to noxious thermal, electrical, and mechanical stimuli. These results suggest that dorsal horn neuronal hyperexcitability in inflamed animals is still subject to modulation by descending modulatory systems. It also suggests that a portion of dorsal horn hyperexcitability may be developed through an intrinsic spinal mechanism. A clinically available N-methyl-D-aspartate (NMDA) receptor antagonist, dextrorphan, and an neurokinin 1 (NK1) receptor antagonist, WIN 51708, were used to analyze the interaction between two receptor systems in hyperalgesia. An isobolographic analysis revealed that the ED50 values for attenuating thermal hyperalgesia, obtained from co-administration of the drugs at 1:9, 1:1.44 and 9:1 ratios (dextrorphan:WIN 51,708, by weight), were not significantly different from the isobolographic line generated by individual ED50 values of the two receptor antagonists. These results suggest that although both NMDA and NK1 receptor systems play a role in inflammation induced hyperalgesia, the two systems appear to act independently. We studied neurochemical changes in rats that had chronic constriction injury (CCI) of the sciatic nerve and received nerve growth factor (NGF) infusion via an osmotic pump. There appeared to be an increase in substance P-like immunoreactive staining in the spinal dorsal horn and lumbar dorsal root ganglion in NGF-treated rats as compared to saline treated rats. The reduction of substance P-like immunoreactivity (LI) in the dorsal horn after CCI of the sciatic nerve was less clear or abolished in rats that received NGF infusion. The induction of NPY in CCI rats was not affected by NGF treatment. The somatostatin and CGRP-LI in the dorsal horn and lumbar dorsal root ganglion did not appear to be affected by NGF treatment. These results suggest that NGF may produce its effects in CCI rats by modulating the substance P content in the spinal cord and dorsal root ganglion. These studies characterize physiological, pharmacological and neurochemical mechanisms that contribute to the development of spinal hyperexcitability and behavioral hyperalgesia in animal models of peripheral nerve or tissue injury. A better understanding of these mechanisms may lead to improved treatment of chronic pain.







DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00509-05 NAB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms of Neuropathic Pain in Humans

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Gracely, Richard H.	Research Psychologist	NA NIDR
Others: Bennett, Gary	Chief, NPPMS	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR
Max, Mitchell	Chief, CPU	NA NIDR
Smith, Wendy	Psychologist	NA NIDR

COOPERATING UNITS (if any)

Turner, Maria	Dermatologist	NCI
---------------	---------------	-----

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neuropathic Pain Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.89

PROFESSIONAL:

0.55

OTHER:

0.34

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project uses both patients and normal volunteers to investigate the activation and maintenance of mechanisms responsible for central enhancement of spontaneous and evoked pain. Previous findings with patients with peripheral neuropathic pain resulted in a model in which persistent input from nociceptor afferents dynamically maintained a central process resulting in wide spread spontaneous pain, allodynia (pain to light touch) secondary hyperalgesia (enhanced pain response to a noxious stimulus) and other sensory and motor abnormalities. One patient in this previous study returned with increased pain in her leg described as a "deep cold pain". Replication of a previously successful block of her injury site alleviated all pain except for this cold pain, suggesting the possibility of an independent pain mechanism that is not dynamically maintained by input from the injury site. Our previous model, derived from patients with neuropathic pain, proposed that maintaining input from pain afferents could arise from either neuropathic sources (damage to the fibers) or from normal nociception in undamaged fibers. In collaboration with the National Cancer Institute, our methods were applied to a population in which the maintaining input was presumed to be from inflammation. Four patients with persistent localized inflammatory disease were examined and particularly painful foci (4 to 5) were each injected with 0.1 to 0.2 ml of 2% lidocaine. During the 20 min of anesthesia, spontaneous pain was reduced or abolished, and touch applied to previously painful, unanesthetized areas was perceived as touch and not as pain. This result further confirms our model of altered central processing maintained by input from nociceptors and supports the concept that the variety of syndromes may reflect the various means by which this input may be achieved. Previous findings with intradermal injections of capsaicin in normal volunteers have supported the use of this model as an analog of clinical neuropathic pain, and formed the basis of a new project examining the influence of NMDA receptor antagonists on the consequences of capsaicin injection, i.e. extent and magnitude of both allodynia (pain to light touch) and secondary hyperalgesia (enhanced pain to pinprick). An additional study is investigating the observation that after the spontaneous pain of capsaicin has waned, it can be rekindled by several interventions including partial or complete occlusion of circulation by a blood pressure cuff, or by partial arterial occlusion by manual palpation. Findings this year have shown that this rekindled pain is not likely due to direct pressure on nerves, and that the manipulations that reproduce the spontaneous pain also abolish cutaneous blood flow. These results provide evidence for the rekindled pain but do not distinguish between central mechanisms and peripheral mechanisms such as reduced clearance of an algescic substance. These studies will continue, and several additional studies will examine the duration of pain sensations produced by heat applied to the zone of allodynia, the effect of trains of thermal and electrical stimulation applied to this zone, and the use of the RIII nociceptive reflex as a measure of central spinal summation. New routes of administration, such as intramuscular capsaicin, and topical capsaicin and mustard oil will be evaluated to 1) investigate mechanisms of deep pain, 2) provide baseline data for brain imaging studies, and 3) develop more consistent models for trials of therapeutic agents.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00532-04 NAB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pathophysiology of Chronic Pain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: DeNucci, Donald	Visiting Scientist	NA NIDR
Others: Dionne, Raymond	Research Pharmacologist	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR
Gracely, Richard	Research Psychologist	NA NIDR

COOPERATING UNITS (if any)

Frank, Joseph	Director, NMR Center	DRD, CC
Rosenbaum, Lola	Physical Therapist	DRM, CC

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Nociception and Tissue Injury Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.35

PROFESSIONAL:

1.25

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This project is attempting to better characterize the pathophysiology of chronic facial pain through a series of clinical investigations. A previous study demonstrated the sensitivity, reproducibility, and utility of pain pressure thresholds for identifying painful muscles in patients with temporomandibular disorders (TMD). This device was then used as a dependent measure in an evaluation of iontophoretic application of a steroid to patients with painful temporomandibular joints (TMJ) as a method for achieving high therapeutic levels of drugs in the TMJ without systemic administration. A standard drug and dose normally administered by iontophoresis, 0.4% dexamethasone in a 4% lidocaine vehicle, was compared to saline placebo. Both dexamethasone and placebo produced a significant reduction in pain scores from baseline following the first two treatments. No difference, however, was seen between groups for pain report or mandibular range of motion. This observation suggested that iontophoretic drug administration to the TMJ may not be an effective method for administering investigational agents or prototypic drugs to investigate the pathophysiology of chronic facial pain originating from the TMJ and associated structures. A current study is evaluating the direct injection of local anesthetic into a nerve which provides innervation to the TMJ with measurement of pain pressure thresholds to determine if this route of administration is a more useful route of administration for investigational interventions. An additional current study is evaluating the relationship between sleep and temporomandibular disorders which is thought to result from nocturnal muscle hyperactivity leading to pain in the muscles of mastication, especially upon awakening. A double-blind crossover study is being conducted in which subjects receive either triazolam, a benzodiazepine hypnotic, or placebo over the course of four days with concurrent monitoring of pain and sleep architecture. Following a three day washout period, subjects then receive the alternative treatment and are monitored similarly. Documentation of an improvement in the quantity and quality of sleep by polysomnography and a parallel change in pain in the temporomandibular region will be interpreted as evidence of a relationship between sleep disorders and orofacial pain.







DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00556-03 NAB

PERIOD COVERED

October 1, 1994 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neuropeptide Interactions with Excitatory Synapses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Caudle, Robert M.	Staff Fellow	NA NIDR
Others: Dubner, Ronald	Chief, NAB	NA NIDR
Ho, Janice	Summer Intern	NA NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Nociception and Tissue Injury Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.1

PROFESSIONAL:

1.0

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The primary focus of this project during the past year was the physiological action of dynorphin, an opioid peptide, on excitatory amino acid receptor function. Whole cell voltage clamp experiments carried out in the CA3 region of the guinea pig hippocampal slice preparation demonstrated that both applied dynorphin and dynorphin released from the tissue itself bind to, and activate, kappa2 opioid receptors. The activation of the kappa2 receptors results in a pronounced and sustained inhibition of the N-methyl-D-aspartate (NMDA) subclass of excitatory amino acid receptors. The NMDA receptors are an extremely important class of receptors that are involved in normal synaptic transmission, learning and memory, and in many pathophysiological states. In our laboratory, it was demonstrated that NMDA receptors are involved in the maintenance of persistent pain. Receptor binding studies indicate that kappa2 receptors in the rat spinal cord are localized to the same region as the NMDA receptors. Preliminary results in rats indicate that spinally administered kappa2 agonists reduce hyperalgesia in a manner similar to NMDA receptor antagonists. This knowledge about kappa2 opioid receptors represents a significant advance for the treatment of chronic pain and other diseases involving NMDA receptors. Future studies on kappa2 receptors will be directed toward understanding the mechanism by which the kappa2 receptor and the NMDA receptor are coupled. We will also complete the behavioral studies to verify the potential clinical utility of agonists for kappa2 receptors in the treatment of chronic pain. In addition to acting at kappa2 opioid receptors, dynorphin also binds directly to the NMDA receptor complex at a non-opioid site. We demonstrated that dynorphin applied to the guinea pig hippocampal slice in low concentrations enhances the activity of NMDA receptors through this site. Future studies on the non-opioid binding site for dynorphin will focus on further characterization of the pharmacology of dynorphin at this site.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00599-02 NAB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Somatosensory Studies of Pain and Pain Control Measured with PET and Functional MRI

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Iadarola, Michael J.	Research Pharmacologist	NA NIDR
Others: Coghill, Robert	IRTA Fellow	NA NIDR
Max, Mitchell B.	Chief, Clinical Trials Unit	NA NIDR
Kenshalo, Jr., Daniel R.	Research Biologist	NA NIDR
Bennett, Gary J.	Chief, NPPM Section	NA NIDR
Gracely, Richard	Research Psychologist	NA NIDR

COOPERATING UNITS (if any)

Berman, Karen F. Chief, PET Imaging Unit CBDB NIMH  
Laboratory of Cardiac Energetics, National Heart, Lung and Blood Institute

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Nociception and Tissue Injury Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.5

PROFESSIONAL:

1.4

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this clinical research effort is to understand how the human central nervous system processes pain information and to identify abnormalities in patients with neuropathic and/or chronic pain conditions. High resolution (a) positron emission tomography (PET) with various radiotracers or (b) functional magnetic resonance imaging (fMRI) techniques are used to assess regional brain activity via blood flow changes. FMRI provides imaging of activity in single transaxial slices in very rapid temporal sequences and PET provides imaging in multiple slices encompassing the entire brain simultaneously. These methods allow us to employ state of the art functional and structural brain imaging techniques to the study of normal and pathological pain states. Over the past year we have conducted oxygen-15 water blood flow PET studies on 3 normal subjects and 4 patients with chronic pain (1 post mastectomy, 3 unilateral post-herpetic neuralgia) and fMRI studies on numerous normal subjects and one neuropathy patient. Analysis of imaging data requires the use of a dedicated computer workstation and one of our main activities has been to establish our own workstation, and data archiving systems. Bringing the computer on-line eliminates one of the major bottlenecks to the imaging program, and we can now implement image analysis programs from DCRT; the Analyze package of programs, the Statistical Parametric Mapping program, and other programs developed by the NIH imaging community. Incorporation of an optical disk drive has facilitated data archiving. One of the most interesting PET observations we had made was that chronic pain patients exhibited a decreased blood flow in the contralateral thalamus. We have extended this to several patients with post herpetic neuralgia (PHN) and the thalamic asymmetry continues to be observed. We now have the ability to register the PET data to high resolution MRI scans which may permit us to localize the decreases to specific thalamic nuclei. These data suggest that the abnormality extends across diagnostic categories and may be a characteristic feature of neuropathic pain states. We have extended the fMRI studies from single slice to multislice slice acquisitions and are currently investigating the influence of increasing levels of activation using motor tasks on the spatial distribution of the fMRI signals. The data suggest that different levels of movement result in increased signal intensity but the spatially discrete nature of the activation signal is retained. Thus the method is likely to be sensitive to spatial aspects of pain processing within primary sensory cortex. FMRI provides a powerful new tool for investigation of dynamic aspects of human pain and its disorders.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00600-02 NAB

PERIOD COVERED

October 1, 1993 to May 1, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Analysis of the Neuronal Responses to Pharmacological Treatments

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Besse, Dominique

Visiting Fellow

NA NIDR

Others: Ruda, Maryann

Chief, CMMS

NA NIDR

Ren, Ke

Visiting Associate

NA NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Cellular and Molecular Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.87

PROFESSIONAL:

0.87

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

These studies are designed to investigate the regulation of gene expression following pharmacological treatments that alter the response to noxious stimulation of the periphery. We are particularly interested in gene regulation occurring in the dorsal root ganglia (DRG) which contain the cell bodies of primary afferent neurons, and in the spinal cord, where the first inhibitory modulation of nociception occurs. Two series of studies have been initiated. In the first series of experiments, using RNA blot analysis of the spinal cord, morphine treatment was found to inhibit the inflammation-induced increase in c-fos mRNA, prodynorphin and, to a lesser extent, proenkephalin mRNAs. These preliminary studies provide evidence that this methodology can be used to examine pharmacological effects on gene expression. The morphine-induced reduction of opioid mRNA expression after inflammation-induced hyperalgesia further support an action of morphine at spinal levels. The parallel decrease in c-fos mRNA provides additional support for a relationship between Fos transcription factor and opioid mRNA regulation. The second series of experiments addressed chronic morphine treatment and the induction of tolerance. Morphine often remains the only drug able to relieve persistent pain although the risk of tolerance is a major clinical concern. Thus, it is important to understand the mechanism of such phenomena. Our initial experiments looked at the effects of morphine and the morphine antagonist naltrexone on transcription factor mRNA regulation in the DRG. In rats treated intrathecally with morphine (30 µg/kg/hr) for 4 days, RNA blot analysis identified an initial up-regulation in the expression of c-jun mRNA compared to saline-treated rats. Behaviorally, the animals were hypoalgesic to a radiant heat stimulus. In morphine tolerant rats, naltrexone administration produced withdrawal and led to an increase in c-jun mRNA expression in the DRG. These data, suggest that some genes responding to Jun transcription factor may have their expression regulated in response to long term morphine administration and withdrawal. Such changes could be part of the mechanism or the consequence of tolerance to morphine. This project has been terminated and the findings are being prepared for publication.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00614-01 NAB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Analysis Of Genes Regulated During Neuronal Injury And Regeneration

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: MacArthur, Linda	Staff Fellow	NA NIDR
Others: Ruda, Maryann	Chief, CMMS	NA NIDR
Caudle, Robert	Staff Fellow	NA NIDR
Allen, Barbara	Biologist	NA NIDR
Franklin, Emma	Biological Lab Tech.	NA NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Cellular and Molecular Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objective of this research program is to clone novel genes involved in the repair of the central and peripheral nervous systems. Model systems which demonstrate repair of the spinal cord are being introduced. The salamander model is an example of functional repair of the spinal cord following complete transection at the cervical, thoracic, or lumbar region. Recovery of function is due to the establishment of the original circuitry. The rat embryo model is an example of limited spinal cord repair in a mammalian system. Embryonic spinal cord transplants, days E14-16, placed into a damaged spinal cord of a neonate result in the recovery of normal walking and climbing behaviour in adult animals. Furthermore, embryonic spinal cords which are lesioned and cultured in vitro survive and grow neurites through the lesion site. Therefore, the potential for CNS repair in a mammal exists at an early developmental stage. A third model system, for peripheral nerve regeneration, will be used to examine the up-regulation of genes in the dorsal root ganglia following sciatic nerve transection. Neurons in the dorsal root ganglia are the first level of processing of neuronal information from the periphery. It has been speculated that genes induced after axotomy of the sciatic nerve are involved in nerve regeneration. Several different cDNA screening strategies are being used to identify novel genes expressed following injury. Two polymerase chain reaction (PCR) based methods, differential display and arbitrarily primed PCR, are being used, and, as an alternate strategy, we are using standard subtraction cloning methods. Using modifications of the differential display method we have isolated clones that appear to be regulated and are currently determining whether the regulation can be verified by using Northern analysis.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00464-07 ASHAB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Natural History of Oral Manifestations of HIV Infection

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Winn, Deborah M.	Chief, ASHAB	EODPP, NIDR
Nowjack-Raymer, Ruth	Public Health Res. Spec., DPS, DPHPB	EODPP, NIDR
Brunelle, Janet A.	Statistician, ASHAB	EODPP, NIDR
Kaste, Linda M.	Senior Staff Fellow, ASHAB	EODPP, NIDR
Rams, Thomas	Staff Fellow, DPS, DPHPB	EODPP, NIDR
Kleinman, Dushanka	Acting Director	NIDR

COOPERATING UNITS (if any)

Walter Reed Army Institute of Research

LAB/BRANCH

Analytical Studies and Health Assessment Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.65

PROFESSIONAL:

.40

OTHER:

.25

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

United States military personnel and dependents who have tested seropositive for the Human Immunodeficiency Virus (HIV) are given medical examinations and treatment at Walter Reed Army Medical Center. Subjects are also invited to participate in a research protocol to study the natural history of HIV infection, conducted by the Walter Reed Army Institute of Research. An oral health research component conducted by NIDR is a part of this natural history study.

The oral component documents the prevalence and incidence of oral pathologic conditions in relation to the stage of HIV infection and systemic disease. Risk factors associated with these conditions are also characterized, and the role of oral manifestations as early predictors or markers of disease progression are studied. Areas of emphasis are mucosal diseases, periodontal conditions, candidal infections, and salivary constituents.

Field data collection for this study ended in August 1994. Approximately 814 persons were seen at least once. About 40% of these persons have had 3 or more exams and 13% had at least 5 exams. Computerization of the biological specimens files, data entry, data editing, coordination of external requests for collaboration on analyzing biological specimens, and development of analytical plans have occurred this year.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00496-06 ASHAB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Determinants of Permanent Tooth Loss in Connecticut and North Carolina

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Marcus, Stephen E.	Sr. Epidemiologist, HAS, ASHAB	EODPP, NIDR
Brown, L. Jackson	Director	EODPP, NIDR
Snowden, Cecelia	Chief, HAS, ASHAB	EODPP, NIDR
Winn, Deborah M.	Chief, ASHAB	EODPP, NIDR
White, B. Alexander	Sr. Dent. Res. Investigator, AES, ASHAB	EODPP, NIDR
Redford, Maryann	Public Health Spec.	EODPP, NIDR

COOPERATING UNITS (if any)

University of Connecticut, Farmington, Connecticut

LAB/BRANCH

Analytical Studies and Health Assessment Branch

SECTION

Health Assessment Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.43

PROFESSIONAL:

0.41

OTHER:

.02

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objective of this study is to measure permanent tooth loss and the factors which influence it. The study will be conducted in two (2) phases. The first phase will be independent of the second and will be a complete study without the second phase. Specific aims of Phase I are to describe 1) the biological condition of extracted teeth, 2) the sociodemographic, attitudinal, economic, and dental care-seeking characteristics of individuals who have extractions, and 3) selected characteristics of the dental providers who perform the extractions. Phase II will be conducted after the first phase and will collect information on patients whose teeth were treated with dental services that are alternatives to extraction for given biological conditions. These teeth will be controls for the extracted teeth and will allow the estimation of a model which explains the factors which influence the choice between extraction and its alternatives. The same practices will be used for both phases. Data from both Phases will be used to develop a more complete explanation of the relative significance of these factors for tooth loss.

Phase I of the project has been completed. Planning for Phase II is nearing completion. The study protocol has been redesigned based on Phase I experience. Although the design for Phase II remains case-control, there have been several modifications: chief among them that a new set of extraction patients will be enrolled into the study and eligibility criteria will be based on the treatments patients receive. Moreover, this redesign includes revised data collection forms (and the addition of a new log form which will collect information about all patients seen by the participating dentist), two new procedures manuals, revised sampling strategy for dentists and patients, and a revised number of patients each dentist is expected to enroll into the study. The new protocol is more complex than that used to guide Phase I or intended for Phase II; increased training and monitoring/support from the program office in each site, however, should ensure high quality data collection.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00504-05 ASHAB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Statistical Analysis of the 1986-87 and 1979-80 NIDR Surveys of School Children

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Snowden, Cecelia B.

Chief, HAS, ASHAB

EODPP, NIDR

COOPERATING UNITS (if any)

Westat Inc.

Rockville, Maryland

LAB/BRANCH

Analytical Studies and Health Assessment Branch

SECTION

Health Assessment Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.42

PROFESSIONAL:

.02

OTHER:

.40

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

During 1986-87 the National Survey of Oral Health in School Children was conducted by Westat Inc. in cooperation with NIDR to monitor the prevalence of oral diseases in school children, grades K-12, throughout the contiguous United States and Hawaii. The 1986-87 survey was a replication and extension of the NIDR National Dental Caries Prevalence Survey conducted in 1979-80, also by Westat, which established baseline estimates on the prevalence of dental caries, gingivitis and dental restorative treatment needs. Both surveys utilized multi-stage probability samples of over 39,000 school children enrolled in grades K-12 to represent over 43 million children enrolled in public or private schools in the seven geographic regions of the U.S. In the 1986-87 survey, additional assessments were made for dental fluorosis, soft tissue lesions, and the use of smokeless tobacco. Residential histories, health and demographic data were collected for each child participating in the clinical examination.

The objective of this ongoing collaborative effort is to develop generalized variance models for selected non-binary statistics common to both surveys, to calculate correlations between the two surveys and to measure design effects in order to establish optimal cluster sizes for future national surveys.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 DE00527-04 ASHAB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Software for Analyzing Data From Complex Dental Surveys

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Snowden, Cecelia B.

Chief, HAS, ASHAB

EODPP, NIDR

COOPERATING UNITS (if any)

National Center for Health Statistics, Center for Disease Control  
Hyattsville, Maryland

LAB/BRANCH

Analytical Studies and Health Assessment Branch

SECTION

Health Assessment Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.09

PROFESSIONAL:

.01

OTHER:

.08

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects   ☐ (b) Human tissues   ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

The development of a data analysis software package (SUDAAN) that is appropriate for the analysis of complex sample surveys is an ongoing project of the National Center for Health Statistics through a contract with Research Triangle Institute (RTI). SUDAAN is a collection of several high level statistical procedures that employ state-of-the-art methodology, Taylor series or Delta method of estimation for analyzing data from complex sample survey designs. Data from two Children's, Adults and Seniors Dental Surveys are being used to analytically test and evaluate SUDAAN in five areas:

- (1) appropriate methodology for complex survey samples
- (2) portability
- (3) reliability/numerical accuracy
- (4) computational efficiency
- (5) ease of modification/enhancement

Work is proceeding in these areas with SUDAAN on the mainframe.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00542-04 ASHAB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Tooth Loss Among the Elderly in the United States

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Marcus, Stephen E.	Senior Epidemiologist, HAS, ASHAB	EODPP, NIDR
Kaste, Linda M.	Senior Staff Fellow, AES, ASHAB	EODPP, NIDR
Brown, L. Jackson	Director	EODPP, NIDR
Winn, Deborah M.	Chief, ASHAB	EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Analytical Studies and Health Assessment Branch

SECTION

Health Assessment Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.38

PROFESSIONAL:

.36

OTHER:

.02

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objectives of this study are to characterize the trends in tooth loss in the U.S. population and among major sociodemographic groups using data from repeated cross-sectional surveys and to model risk factors for tooth loss using longitudinal data from panel studies. Data from Sri Lanka and Norway were used to develop appropriate measures and test analytical strategies. Plans call for further analyses of the VA longitudinal data base and repeated cross-sectional surveys of the U.S. population.

This year, data from the National Survey of Oral Health in U.S. Employed Adults and Seniors: 1985-1986 (conducted by the National Institute of Dental Research) were analyzed to examine the prevalence and demographic correlates of tooth loss among the elderly (overall n = 5,649 persons aged 65+ years attending senior centers). Results show that there were important differences in tooth loss among subgroups of the elderly sample. The oldest seniors and those with the least education or income were the most likely to be edentulous. The oldest dentulous seniors, blacks, those with the least education or income, and those who lived in New England of the Northeast had the fewest number of teeth present.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00544-04 ASHAB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Periodontal Health in Adolescent Americans

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Brunelle, Janet A.	Statistician (Health), AES, ASHAB	EODPP, NIDR
Brown, L. Jackson	Director	EODPP, NIDR
Albandar, Jasim	Visiting Scientist	EODPP, NIDR
Winn, Deborah M.	Chief, ASHAB	EODPP, NIDR
Oldakowski, Richard	Chief, SPU, ASHAB	EODPP, NIDR

COOPERATING UNITS (if any)

Westat, Inc.; University of Minnesota; University of Tennessee; SUNY, Buffalo; Columbia University; VPI; Medical College of Virginia

LAB/BRANCH

Analytical Studies and Health Assessment Branch

SECTION

Analytical Epidemiology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.22

PROFESSIONAL:

.20

OTHER:

.02

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A major contract to relocate, re-examine and collect risk factor information on children who were classified as having LJP, GJP or incidental LA in the 1986-87 survey began in October, 1991. Research objectives are to: a) assess the progression of periodontal destruction among the cases of early onset periodontitis, b) characterize the microbial ecology of the sub-gingival plaque among persons with early onset periodontitis, and c) compare the presence and concentration of selected putative pathogens and high-resistance factors among individuals with early onset periodontitis to controls.

A sample of all cases whose oral examinations from the 1986-87 survey indicated early onset periodontitis or other severe periodontal problems was selected for study. Two controls per case matched by age, gender, race and geographic location were also located and invited to participate in the study. During FY'93, examinations were made on approximately 265 young people 19-25 years of age. Full mouth oral examinations for periodontal measures and dental caries were conducted. Biological specimens, blood, gingival crevicular fluid and subgingival plaque fluid were also collected. A questionnaire including such variables as medical history, family history, and dental utilization was administered at the time of the oral exam.

Dental examination data was edited and summary measures derived. Laboratory analyses of serum, plaque and gingival crevicular fluid were conducted by a number of different laboratories. Relationships among the many clinical and laboratory variables measured are being evaluated.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00567-03 ASHAB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Oral Physiology of Aging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Streckfus, Charles F.	Sr. Staff Fellow, AES, ASHAB	EODPP, NIDR
Brown, L. Jackson	Director	EODPP, NIDR
Brunelle, Janet A.	Statistician (Health), AES, ASHAB	EODPP, NIDR
Oldakowski, Richard A.	Chief, SPU, ASHAB	EODPP, NIDR
Kingman, Albert	Senior Statistician, OD	EODPP, NIDR

COOPERATING UNITS (if any)

NIA, FSK/JHU

LAB/BRANCH

Analytical Studies and Health Assessment Branch

SECTION

Analytical Epidemiology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.02

PROFESSIONAL:

.95

OTHER:

1.07

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects   ☐ (b) Human tissues   ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The oral research component of the Baltimore Longitudinal Study of Aging (BLSA), since its inception in 1978, has been designed to evaluate the physiological and pathological factors that influence the oral health and function of individuals of different ages. Currently, the EODPP, is developing plans to broaden the scope of research to include studies of alveolar bone loss in the oral cavity, the detection and application of oral molecular biological markers for systemic disease, and an expanded periodontal evaluation implementing protein markers and DNA microbial probes for early disease detection. The oral epidemiology component is also working with the BLSA to increase minority enrollment thereby increasing the diversity of the BLSA population base. The implementation of these additional areas of investigation within the BLSA, present an opportunity to enhance the overall understanding of age related changes in the oral cavity.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00572-02 ASHAB

PERIOD COVERED

October 1, 1993 - September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cost and Utilization of Dental Services - National Medical Expenditure Survey

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

White, Benjamin A.	Sr. Dent. Res. Investigator, AES, ASHAB	EODPP, NIDR
Kaste, Linda M.	Sr. Staff Fellow, AES, ASHAB	EODPP, NIDR
Marcus, Stephen E.	Senior Epidemiologist, HAS, ASHAB	EODPP, NIDR
Oldakowski, Richard	Chief, SPU, ASHAB	EODPP, NIDR
Zion, Gary	Computer Programmer, HAS, ASHAB	EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Analytical Studies and Health Assessment Branch

SECTION

Analytical Epidemiology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.33

PROFESSIONAL:

.33

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In 1987, the National Medical Expenditure Survey (NMES) II was conducted by the Center for General Health Services Intramural Research, Agency for Health Care Policy and Research. The survey provides extensive information on health expenditures by or on behalf of American families and individuals, the financing of these expenditures, and each person's use of services during the period from January 1 to December 31, 1987. The NMES II Household Survey is based on a national probability sample of the civilian, noninstitutionalized population living in the community. The sample is designed to provide a larger representation of population groups of special policy interest to the Federal Government than would have been obtained from a random sample. These groups include poor and low income families, the elderly, the functionally impaired, and black and Hispanic minorities.

As part of NMES II, information was collected on the type of dental services provided, total dental expense, and sources of payment for all dental services. This project uses these data to examine several issues related to dental services, such as: 1) overall dental utilization and expenditure patterns; 2) geographic variation in practice patterns for dental services; 3) demographic and socioeconomic variation in use of dental services; 4) associations between the use of dental services and other health care services; 5) relationship between the use of dental services and reported oral and general health status; 6) use of dental services by Native Americans and Alaska Natives; 7) use of dental services and health related behaviors, including care seeking and preventive care; 8) usual source of medical and dental care and reasons for lack of a usual source of dental care; and 9) health insurance status and use of dental services.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00573-02 ASHAB

PERIOD COVERED

October 1, 1993 - September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Patterns of Care, Outcomes, and Cost of Oral Cavity and Pharyngeal Cancer

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

White, B. Alexander	Sr. Dent. Res. Investigator, AES, ASHAB	EODPP, NIDR
Winn, Deborah	Chief, ASHAB	EODPP, NIDR
Kleinman, Dushanka	Acting Director	NIDR

COOPERATING UNITS (if any)

Applied Research Branch, Surveillance Program, Division of Cancer Prevention and Control, National Cancer Institute and the Division of Beneficiary Studies, Office of Research, Health Care Financing Administration

LAB/BRANCH

Analytical Studies and Health Assessment Branch

SECTION

Analytical Epidemiology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.25

PROFESSIONAL:

.23

OTHER:

.02

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects   ☐ (b) Human tissues   ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

EODPP has been working with the National Cancer Institute (NCI) to obtain the SEER/Medicare Linkage project database for use in a number of investigations in oral cancer. The Linkage Project is a collaborative effort by the NCI and the Health Care Financing Administration (HCFA) to link the NCI's Surveillance, Epidemiology, and End Results (SEER) Program data base, which contains information on cancer cases diagnosed and reported in nine geographically distinct population-based tumor registries, and HCFA's Medicare statistical system (MSS), which contains extensive billing information for the health care of the disabled and more than 95 percent of the elderly.

Initial work has begun at NIDR to developing an analysis plan for oral cavity and pharyngeal cancer utilizing this linked data base. The objectives of this study are to: 1) identify regional (SEER) variations in the choice of first-course of cancer directed therapy among individuals 65 years of age and over with oral cavity and pharyngeal cancer, by site of cancer; 2) add to the information currently available concerning the effectiveness of alternative therapies used to manage oral cavity and pharyngeal cancer among the elderly population; and 3) estimate the lifetime costs associated with oral cavity and pharyngeal cancer.

Based on the analysis plans, data has been obtained on the choice of first-course of cancer directed therapy and on initial costs associated with oral cavity and pharyngeal cancer.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00579-02 ASHAB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Dental Caries and Selected Microbiological Determinations in Hispanic Children

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Brunelle, Janet A.

Statistician (Health), AES, ASHAB

EODPP, NIDR

Oldakowski, Richard

Chief, SPU, ASHAB

EODPP, NIDR

COOPERATING UNITS (if any)

Stanley B. Heifetz

University of Southern California

Jorgen Slots

University of Southern California

LAB/BRANCH

Analytical Studies and Health Assessment Branch

SECTION

Analytical Epidemiology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.16

PROFESSIONAL:

.11

OTHER:

.05

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

As part of a sealant program being conducted in inner city schools in Los Angeles, approximately 190 Hispanic children in grades 2 and 3 (7 and 8 years of age), were selected for inclusion in a microbiology-carries study. One clinical examiner scored caries (dmfs) on deciduous teeth and DMFS on permanent teeth. Before the clinical exam, children rinsed with 5 ml sterile distilled water for 15 seconds to provide microbiological samples. Questionnaires to check residency and heritage information were also obtained.

All samples were delivered to the laboratory on same day and plated on TYCSB agar for mutans streptococci and Rogosa SL agar for Lactobacillus. Total viable counts (CFU/ml of salivary rinse) of S. mutans, S. sobrinus and Lactobacillus species were determined for each child. Analyses of the associations between microbiological counts of S. mutans, S. sobrinus and Lactobacillus and deciduous and permanent caries status were made. For deciduous caries, distributions of children by presence or absence of caries and 'none' or 'any' bacterial counts were made.  $\chi^2$  tests of these distributions indicated strong associations between dmfs and both mutans streptococci and Lactobacillus ( $p < .001$ ). Mean dmfs scores (standard errors) for 'none' and 'any' mutans streptococci were 1.61 (0.6) and 8.05 (0.6) respectively and 3.88 (0.8) and 9.36 (0.7) for Lactobacillus, respectively. Mean caries levels increased as counts of mutans streptococci or Lactobacillus increased.

These data reaffirm the strong association between dental caries in the deciduous dentition and mutans streptococci and lactobacillus. Further analysis of bacterial counts, dental caries and acculturation are being made.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00580-02 ASHAB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Occupation and Reproductive Health of Women Dentists

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Kaste, Linda M.

Sr. Staff Fellow, AES, ASHAB

EODPP, NIDR

Doan, Linh

Junior Programmer, SPU, ASHAB

EODPP, NIDR

COOPERATING UNITS (if any)

American Dental Association, University of North Carolina, and NIEHS

LAB/BRANCH

Analytical Studies and Decision Systems Branch

SECTION

Analytical Epidemiology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.50

PROFESSIONAL:

.30

OTHER:

.20

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A national survey of occupational exposures assessing the relationships of the practice of dentistry and the reproductive health of women dentists was conducted via a mailed questionnaire. Approximately 5,500 women who graduated from dental school from 1977 to 1986 and were 31-40 years old in the spring of 1992 had been mailed questionnaires. The sample was chosen to allow for the opportunity to have dental occupational exposures during reproductive ages. The methodology builds upon work conducted at NIEHS on occupational exposures focusing on amalgams and nitrous oxide and primarily the reproductive outcomes of time to pregnancy and spontaneous abortion. This is the first attempt for the acquisition of these types of data via self administered questionnaire. An initial mailing, post-card follow-up and thank you, second questionnaire, and a follow-up letter from the American Dental Association, for a total of four mail contacts were made during fiscal year 93. FY94 involved an additional mailing via registered mail. A response rate near 70 percent has been achieved. Data entry was completed and data editing, and analysis have begun.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00581-02 ASHAB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biomarkers for Oral Cancer

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Winn, Deborah M.

Chief, ASHAB

EODPP, NIDR

Schwartz, Joel L.

Section Chief, BDMS, MEDIB

EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Analytical Studies and Health Assessment Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.23

PROFESSIONAL:

.21

OTHER:

.02

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Recent laboratory developments have identified biomarkers measurable in human fluids or tissues with may help further our understanding of the process of carcinogenesis and identify persons at especially high risk of oral cancer. Among these are p53 tumor suppressor genes and heat shock proteins. This project will examine biomarkers in a cohort of persons with oral premalignant lesions who are monitored for changes in biomarker status changes in histopathology, or the development of cancer. This year considerable progress was made in development of the protocol. A contract to support the field data collection was awarded. Negotiations are on-going with the Veteran's Affairs Administration; the study population will be drawn from VA hospital dental clinics. The next year will see the finalizing of the protocol and the utilization of data collection and laboratory assessments. If the research can identify biomarkers which precede disease in a predictable way, there may be the potential to prevent oral cancer, screen for more successfully, and/or treat it at an earlier stage.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00606-01 ASHAB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Determination of Serum Antibodies to Periodontal Pathogens in the U.S. Population

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Marcus, Stephen E.	Senior Epidemiologist, ASHAB	EODPP, NIDR
Winn, Deborah M.	Chief, ASHAB	EODPP, NIDR
Brown, L. Jackson	Director	EODPP, NIDR
Rams, Thomas	Staff Fellow, DPS, DPHPB	EODPP, NIDR
Snowden, Cecelia	Chief, HAS, ASHAB	EODPP, NIDR

COOPERATING UNITS (if any)

NCHS

LAB/BRANCH

Analytical Studies and Health Assessment Branch

SECTION

Health Assessment Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.37

PROFESSIONAL:

.35

OTHER:

.02

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The study population is comprised of persons aged 13 to 65 examined in Phase I of the Third National Health and Nutrition Examination Survey (NHANES III) (age 13 is the youngest at which periodontal assessments are made in NHANES III). The prevalence of elevated antibody titers to these organisms will be described for the entire sample and stratified by age, gender, race, and geographic region. Moreover, these systemic antibody titers will be correlated to the clinical periodontal status of study participants. Serum from the NHANES III subjects, which has already been collected, frozen and stored for further analysis, will be assayed by ELISA procedures. The ELISA offers the benefit of quantification of antibody titers; values of 100 EU or greater for A.a. and 20 EU or greater for P. gingivalis will be employed as threshold values for designating elevated levels.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00615-01 ASHAB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Methodology and Analytic Development of HANES III

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Winn, Deborah M.	Chief, ASHAB	EODPP, NIDR
Snowden, Cecelia	Chief, HAS, ASHAB	EODPP, NIDR
Oldakowski, Richard	Chief, SPU, ASHAB	EODPP, NIDR
Brunelle, Janet	Statistician (Health), AES, ASHAB	EODPP, NIDR
Kleinman, Dushanka	Acting Director	NIDR
White, B. Alexander	Sr. Dent. Res. Investigator, AES, ASHAB	EODPP, NIDR
Kaste, Linda M.	Senior Staff Fellow, AES, ASHAB	EODPP, NIDR
Streckfus, Charles	Senior Staff Fellow, AES, ASHAB	EODPP, NIDR
Marcus, Stephen E.	Senior Epidemiologist, HAS, ASHAB	EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Analytical Studies and Health Assessment Branch

SECTION

Health Assessment Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.70

PROFESSIONAL:

2.00

OTHER:

.70

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

From 1988 to 1991, the first half of the National Health and Nutrition Examination Survey (NHANES III) was conducted by the National Center for Health Statistics (NCHS). NIDR staff contributed to and coordinated an oral health component comprising parts of the household questionnaires as well as actual dental exams done on selected family members at mobile examination centers.

Interprogram committees were set up to design questions, discuss statistical handling of information and evaluate basic materials. Computer programs to synthesize information, create summary variables and produce distributions to evaluate data were designed and run. Overall plans of analysis for individual oral health measures were designed. Analysis of clinical measures of dental caries, tooth conditions and restorative needs, soft tissue lesions, periodontal disease and orthodontic status was begun. Prevalence distributions by sociodemographic variables and relationships between certain clinical measures were computed and tested. The influence of non-response and medical exclusions on the data were also analyzed. Abstracts and papers for publication were written from this information.

Further analysis of the relationships among oral health variables and other sociodemographic and medical information will be conducted.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 D00616-01 ASHAB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Case Control Study of Oral and Pharyngeal Cancer in Puerto Rico

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Winn, Deborah M.  
Kleinman, Dushanka  
Diehl, Scott

Chief, ASHAB  
Acting Director  
Chief, MEDIB

EODPP, NIDR  
NIDR  
EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Analytical Studies and Health Assessment Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.13

PROFESSIONAL:

.12

OTHER:

.01

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Puerto Rico experiences high rates of oral and pharynx cancer. This study aims to identify sociodemographic, behavioral, nutritional, dental, and occupational risk factors for oral and pharynx cancer in Puerto Rico. Another purpose is to evaluate the role of human papilloma virus and biomarkers for malignancy and malignant transformation in biological specimens from cases and controls. This study has a population-based case-control design with cases ascertained from the cancer registry in Puerto Rico. Cases and controls are interviewed in their homes using a standardized questionnaire which obtains data on a wide range of risk factors. Medical record data is being abstracted on cases. Biological specimens are being obtained from study subjects. Specimens include a blood sample, a buccal cell scraping, a urine sample, and pathologic slides. Data will be analyzed to determine differences between cases and controls in potential risk factors and in the presence and levels of viruses and molecular markers for malignancy.

To date 366 cases and 633 controls have been identified and 251 cases and 451 controls have been interviewed. The overall response rate is very good with refusals accounting for 10% of controls. Only 3 cases have refused to participate thus far, but 39 cases had died by the time of the interview. Biological specimens have been obtained thus far from 32 cases and 76 controls. With collaboration of several academic and commercial laboratories and experts, NIDR and NCI have been evaluating methods for collecting buccal scrapings and laboratory methods for evaluating the presence of human papillomavirus in buccal scrapings. Field work continues on this project.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00618-01 ASHAB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Oral Health Assessment Database Development

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Snowden, Cecelia B.	Chief, HAS, ASHAB	EODPP, NIDR
Oldakowski, Richard	Chief, SPU, ASHAB	EODPP, NIDR
Brown, L. Jackson	Director, EODPP	NIDR
Brunelle, Janet A.	Statistician (Health), AES, ASHAB	EODPP, NIDR
Winn, Deborah M.	Chief, ASHAB	EODPP, NIDR
Webb, Kimberly W.	Statistical Assistant, HAS, ASHAB	EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Analytical Studies and Health Assessment Branch

SECTION

Health Assessment Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.70

PROFESSIONAL:

1.00

OTHER:

.70

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The NHANES III - Phase I, 1988-1991 oral health component database was constructed. Error-checking computer programs minimize errors in the data. Distributions and relationships among variables were produced and analyzed for consistency. Statistical and methodological issues related to integrating data from previous NIDR surveys were also resolved.

The data will be used to contribute to ongoing studies on the magnitude, severity, scope, and interrelationships of oral health conditions in the U.S. population. The documentation and file structure permits researchers to perform their own analyses with minimal technical assistance.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00577-02 DPHP

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Prevention of Dental Caries

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Selwitz, Robert H.	Dental Epidemiologist	EODPP, NIDR
Nowjack-Raymer, Ruth E.	Public Health Research Spec.	EODPP, NIDR
Horowitz, Alice M.	Education Specialist	EODPP, NIDR
Gift, Helen	Chief, DPHP Branch	EODPP, NIDR
Small, John	Public Health Advisor	EODPP, NIDR
Kobayashi, Seigo	Guest Researcher	EODPP, NIDR
Kaste, Linda	Senior Staff Fellow	EODPP, NIDR
Oldakowski, Richard	Chief, SPU, ASHAB	EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Disease Prevention Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.93

PROFESSIONAL:

1.78

OTHER:

.15

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Although dental caries has declined markedly over the past few decades, the diseases still presents a problem for many persons or population groups. By the age of 17, children on average experience nearly eight decayed or filled surfaces; by the ages of 25-29, young adults experience more than 17 decayed or filled surfaces. Dental caries in infants and young children is a significant problem especially among certain racial/ethnic groups in the U.S. DPHPB staff have focused efforts on investigating fluorides and dental sealants, feeding behaviors, and science transfer for enhancing the prevention of dental caries. A study in Nelson County, VA demonstrated the effectiveness of the combined use of fluoride therapy and dental sealants. A background paper for a workshop on guidelines for sealant use reviewed recent changes in the epidemiology of dental caries and assessed their potential impact on the diagnosis and management of the disease and the planning and operation of sealant programs. Analysis of other available data by staff suggest the need to continue efforts toward improving knowledge of oral disease preventive approaches and the proper use of preventive methods. NIDR organized an Ad Hoc working group to review what research activities are being undertaken in the area of infant caries, to identify research needs, and to recommend priorities for future Institute efforts. Several staff members of the Ad Hoc working group also are collaborating with the CDC in a joint effort to chart a course of action at the Federal level to help prevent this condition in young children. In addition, staff are involved in a pilot, community-based study of the prevention of caries among Hispanic infants and young children. Analyses of feeding behaviors data from the 1991 NHIS have been submitted for publication.



PROJECT NUMBER  
Z01DE00577-02 DPHP

Continuation Sheet

Snowden, Cecelia	Statistician	EODPP, NIDR
Zion, Gary	Computer Programmer	EODPP, NIDR
Mercer, Paula	Computer Programmer	EODPP, NIDR





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00578-02 DPHP

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Fluoride Accumulation and Effects in the Body

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Selwitz, Robert H.	Dental Epidemiologist	EODPP, NIDR
Nowjack-Raymer, Ruth E.	Public Health Research Spec.	EODPP, NIDR
Kingman, Albert	Chief Statistician	EODPP, NIDR
Horowitz, Alice M.	Education Specialist	EODPP, NIDR

COOPERATING UNITS (if any)

University of Connecticut, Medical College of Georgia, University of Iowa  
University of Michigan, University of North Carolina at Chapel Hill, and NCHS

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Disease Prevention Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.59

PROFESSIONAL:

.49

OTHER:

.10

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Fluoride accumulation and its effects in the body and interrelations between dental caries, dental fluorosis, and the appropriate use of fluoride are important issues being addressed by staff of the DPHPB. Staff have prepared two manuscripts regarding the assessment of dental fluorosis in child populations having differing levels of exposure to fluoride through drinking water, dietary fluoride supplements, and other sources. A DPHPB staff person served as editor and contributor for the published proceedings of an NIDR sponsored workshop on fluoride accumulation and its effects in the body. The proceedings include a number of recommendations for further research regarding strategies for improving the assessment of fluoride accumulation in body fluids and tissues and strategies for improving the assessment of dental fluorosis. DPHPB staff prepared a paper for presentation at an American Dental Association sponsored workshop on dietary fluoride supplements. In addition, they have engaged in the dissemination of current information regarding the appropriate use of fluorides through papers delivered at professional and other scientific meetings to students, health professionals, and government officials.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00584-02 DPHP

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Minority Oral Health

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Gift, Helen C.	Chief, DPHP Branch	EODPP, NIDR
Robinson, D.	Health Promotion Research Specialist	EODPP, NIDR
Horowitz, Alice M.	Education Specialist	EODPP, NIDR
Kaste, Linda	Staff Fellow	EODPP, NIDR
Rams, Thomas E.	Staff Fellow	EODPP, NIDR
Drury, Thomas	Deputy Branch Chief, DPHPB	EODPP, NIDR
Small, John	Public Health Advisor	EODPP, NIDR
Nowjack-Raymer, Ruth	Public Health Research Specialist	EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Health Promotion Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.70

PROFESSIONAL:

1.60

OTHER:

.10

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Improving oral health in the U.S. requires addressing high risk individuals many of whom are minorities. An RFP for a community demonstration research implementation study was written and issued and the nine responses have been reviewed. An award will be made in FY 1994.

An RFC has been prepared by staff for the NIDR 1996-97 Children's Survey which through an improved sampling strategy will increase information on blacks and Hispanics.

Staff are analyzing NIDR and NCHS data with a specific focus on oral health behaviors and oral health status of Black Americans. These analyses have resulted in presentations at professional meetings and manuscripts for publications. Staff from the Health Promotion Section, in collaboration with a staff member from the Analytical Studies and Decisions Systems Branch, are finalizing an issues paper dealing with Hispanic Oral Health in the United States with special focus on access to care and available clinical disease indicators. This manuscript along with a more extensive outline of Hispanic oral health issues and methodological considerations will be used to develop a proposed research agenda for Hispanic oral health.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00585-02 DPHP

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Prevention of Oral Cancer

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title,

Horowitz, Alice M.

Education Specialist

EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Health Promotion Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.29

PROFESSIONAL:

.19

OTHER:

.10

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Oral soft tissue lesions, precancers and cancers are among the most serious, and in the case of oral cancer life-threatening, oral conditions. The risk factors for many of these conditions are clearly identified as comorbidities with medical conditions and treatments and with high risk behaviors such as tobacco and alcohol use. To that end, these conditions and the risks leading to them are clear targets for health promotion. The Branch is actively pursuing research which identifies correlates of knowledge, opinions and practices associated with these oral conditions and brings focus to intervention research which could improve strategies to reduce the incidence and prevalence of these conditions and associated risk behaviors. Staff are conducting analyses of the 1990 and 1992 NHIS data regarding knowledge of the risks, signs, and symptoms of oral cancer. These analyses were the basis of a manuscript accepted for publication in JADA. Staff are collaborating with a group of researchers at the University of Maryland regarding a statewide study of the knowledge, opinions and practices of the public and health care providers regarding oral cancer. Staff have been active in interagency forums on oral cancer and associated risk behaviors which have been held to identify research and program needs.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00586-02 DPHP

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Women's Oral Health

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Redford, Maryann	Public Health Specialist	EODPP, NIDR
Horowitz, Alice M.	Education Specialist	EODPP, NIDR
Gift, Helen C.	Chief, DPHP Branch	EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Health Promotion Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.16

PROFESSIONAL:

.11

OTHER:

.05

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Women's health has become a major priority for research at NIH. Branch staff have participated with other NIH scientists in developing clinical trial and community demonstration research agendas for women's health. Extensive literature reviews have been conducted to assess the interaction of oral health with systemic health and to determine how oral health fits within the contexts of these larger research initiatives. Projects evaluating the utility of oral biomarkers for systemic diseases and conditions have begun. For instance, saliva is being tested as a matrix for the biochemical validation of fat intake among women enrolled in the Women's Health Trial Minority Feasibility Study. Also, fractal analytical techniques are being applied to radiographs of the jaws and spines in an effort to describe aberrant trabecular bone patterns which may provide an indication of osteoporotic fracture risk.

Another key activity in the area of Women's Health is a study of the oral manifestations of HIV infection in women. Branch staff have taken the leadership role in the development of a comprehensive oral protocol for the Women's Interagency HIV Study.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00587-02 DPHP

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Quality of Life

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Gift, Helen C.

Chief, DPHP Branch

EODPP, NIDR

Redford, Maryann

Public Health Specialist

EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Health Promotion Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.12

PROFESSIONAL:

.12

OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Oral diseases and conditions are highly prevalent and the progressive consequences of these are not only physical, but economic, social, and psychological. The relation of oral health to overall quality of life has gained increasing recognition as an important area of scientific investigation. Branch staff have participated on NIH committees to broaden the depth and scope of quality of life research and have collaborated with other agencies on publications.

This research program represents a series of projects undertaken to conceptualize oral quality of life as well as to describe and then quantify the functional, psychological, and social consequences of oral disorders and their treatment. Extensive literature reviews and consultations with external experts have been conducted in an effort to improve the measurement and interpretation of oral quality of life. Staff have worked with NCHS to improve ways of determining disability in relation to the oral cavity. Based upon a review and synthesis of literature, oral quality of life has been assessed for the specific audience of aging veterans in a position paper for the Department of Veteran's Affairs. This manuscript was further revised and has been accepted for publication.

A major book was co-edited by Branch staff and staff of National Institute on Aging.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00588-02 DPHP

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Bone Loss in the Oral Cavity and its Relation to Skeletal Bone Health

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Redford, Maryann

Public Health Specialist

EODPP, NIDR

Small, John

Public Health Advisor

EODPP, NIDR

COOPERATING UNITS (if any)

National Institute of Arthritis and Musculoskeletal and Skin Diseases  
Bowman Gray School of Medicine

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Health Promotion Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.30

PROFESSIONAL:

.20

OTHER:

.10

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Bone loss in the oral cavity is a significant problem in the United States. In the dentate, oral bone loss may manifest as a loss of tooth support. In edentate individuals, osteopenia may augment local anatomic, biological, and mechanical factors resulting in extensive ridge atrophy. There have been speculations in the medical and dental literature that generalized skeletal osteopenia may be conducive to accelerated loss of oral bone. Thus, skeletal osteopenia may influence the need for and outcome of periodontal, pre-prosthetic, and implant surgical procedures.

This research program represents a series of projects undertaken as follow-up to an NIDR and NIAMS sponsored workshop on osteoporosis and oral bone loss in August, 1992. A staff member served as guest editor to a special supplement to the Journal of Bone and Mineral Research spotlighting the proceedings of the workshop.

In a related activity, staff collaborated with peers from the NIDR and NIAMS extramural programs in developing a Program Announcement to stimulate activity among extramural investigators in accordance with the workshop's research recommendations.

Staff also independently formulated an RFC intended to characterize tooth and oral bone status for a large cohort of pre- and post-menopause women and to correlate this information with skeletal bone density assessments.

Another key activity in this area of research focuses on analyses of data derived from collaboration with scientists at Bowman Gray School of Medicine. Fractal-based indices of trabecular bone patterns from existing dental radiographs were completed and analyses were conducted which indicate that these measures correlate with medically diagnosed osteoporosis.

Staff presented preliminary results of the fractal analysis project at the IADR 1994 meeting. This presentation is being expanded for publication in a peer reviewed journal.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00589-02 DPHP

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Orofacial Trauma

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Gift, Helen C.	Chief, DPHP Branch	EODPP, NIDR
Nowjack-Raymer, Ruth	Public Health Research Spec.	EODPP, NIDR
Witt, Cecilie	Research Assistant	EODPP, NIDR
Bhat, Mohandas	Director, CDDP	EP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Health Promotion Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.30

PROFESSIONAL:

.25

OTHER:

.05

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The consequences of orofacial trauma can be among the most permanent of oral conditions and diseases. Trauma (as a consequence of injuries to the face and mouth resulting from falls, sporting activities or abuse) and prevention of it have recently received more attention in research. Estimates of the number of patients seen in the private dental practice for orofacial trauma have been published. A portion of the 1991 NHIS contained questions about participation of children and youth in sports and their use of head and mouth protection. These data have been analyzed, presented at the 1994 IADR and a manuscript has been submitted for publication.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00590-02 DPHP

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Evaluating Oral Health Status As It Relates to Problematic Eating Behaviors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Redford, Maryann

Public Health Specialist

EODPP, NIDR

COOPERATING UNITS (if any)

University of California, San Francisco

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Health Promotion Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.08

PROFESSIONAL:

.08

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The nutritional status of the elderly has been studied extensively but little is known about how oral health status relates to problematic eating behaviors among nursing home residents. Because of a dearth of information, dentistry is often excluded from inter-disciplinary strategies for treating dysphagia. If oral health problems are identified as contributing to malnutrition, weight loss, or the eventual placement of a feeding tube, then appropriate oral health interventions may improve the quality of life for nursing home residents.

Literature reviews have been conducted to identify research questions and hypotheses. Collaborations have been established with extramural scientists as preparation for investigations in this area. An initial project in collaboration with an extramural scientist is beginning which will examine the role of oral health status in the development of problematic eating disorders among nursing home residents. Existing oral health data will be linked to relevant clinical, social, cultural, and environmental data which are part of a parent study being conducted at the University of California, San Francisco. Interpretation of the results of this study should augment the rationale for improved recognition and treatment of presenting dental needs within this underserved population.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00592-02 DPHP

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Research on Oral Health Education and Health Promotion

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Horowitz, Alice M.	Education Specialist	EODPP, NIDR
Maeda, Naoko	Guest Researcher	EODPP, NIDR
Small, John S.	Public Health Advisor	EODPP, NIDR
Gift, Helen C.	Chief, DPHP Branch	EODPP, NIDR
Nowjack-Raymer, Ruth	Public Health Research Specialist	EODPP, NIDR
Kobayashi, Seigo	Guest Researcher	EODPP, NIDR
Chang, Duk Soo	Guest Researcher	EODPP, NIDR
Snowden, Cecelia	Statistician	EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Health Promotion Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.85

PROFESSIONAL:

2.55

OTHER:

.30

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects   ☐ (b) Human tissues   ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Oral health education is an integral part of health promotion. Research on ways to improve content, channels of distribution, appropriateness as well as the need to identify specific audiences and to identify their needs is a primary focus of the Branch. Science transfer activities to help ensure that research based information is used in preparation of education materials and other communications also is integral to Branch activities. Adapting research findings to the needs of the audience, be they legislative, professional, general public or specific high risk populations is an important outcome of research conducted by NIDR. The Branch takes the NIDR lead in evaluating scientific literature for translation and interpretation for the varied audiences. The outcomes of these scientific evaluations result in published literature reviews in texts and journals, lectures and consultation. Major areas of emphasis are community water fluoridation, multiple modalities of fluoride, dental sealants as well as prevention of other oral diseases and conditions, and strategies for effective health education and promotion.

Staff have been working with outside investigators to develop a survey instrument to evaluate knowledge, opinions and practices for use in determining the basis upon which education and preventive regimens could be established at the local and state levels. Staff also have been working with an outside investigator to evaluate the content of health education text books to determine the extent, nature and appropriateness of oral health information being taught and how this information supports oral health objectives in Healthy People 2000. The Branch is working with three international guest scientists in the areas of health education and health promotion. One is analyzing a survey from Korea on knowledge, opinions, and practices to establish the basis for a health education-promotion program, another is adapting infection control materials for use in Japan.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00593-02 DPHP

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

International Health Promotion, Disease Prevention, and Epidemiologic Research

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Horowitz, Alice M.	Education Specialist	EODPP, NIDR
Nowjack-Raymer, Ruth E.	Public Health Research Specialist	EODPP, NIDR
Kleinman, Dushanka V.	Deputy Director	OD, NIDR
Maeda, Naoko	Guest Researcher	EODPP, NIDR
Kobayashi, Seigo	Guest Researcher	EODPP, NIDR

COOPERATING UNITS (if any)

World Health Organization

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Disease Prevention Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

1.4

OTHER:

.6

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

Numerous international initiatives which focus on improved disease prevention/health promotion research and enhanced science transfer internationally exist and include collaboration with investigators of the WHO International Collaborative Study II; authorship of articles for international publication; participation in international meetings as presenters and invited speakers, and as technical consultants and development of material. Additionally, numerous guest researchers have been hosted by and have collaborated with staff in the development of research protocols for the initiation of studies. Scientific interchange has also been facilitated through the coordination of seminars, lecturers, meetings for international guests and visitors with a broad range of agencies, organizations and universities. Health education materials developed by NIDR have been made available to international organizations and agencies to enhance rapid science transfer.

The subject matter of the international initiatives cover diverse public health topics which include preventive dentistry, dietary fluoride supplements for preschool age children, community water fluoridation, primary prevention of oral disease, infection control, HIV/AIDS prevention and control curriculum development focused on oral disease prevention, role of oral health professionals in oral disease prevention and health promotion.

The World Health Organization (WHO) World Health Day 1994 is oral health. Branch staff were active in developing events for World Oral Health Day and Year.







DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00595-02 DPHP

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Research Toward Preventing Periodontal Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Nowjack-Raymer, Ruth E.

Public Health Research Specialist

EODPP, NIDR

Kingman, Albert

Statistician (Health)

EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Disease Prevention Section

INSTITUTE AND LOCATION

NIDR,NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.28

PROFESSIONAL:

.18

OTHER:

.10

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Self care remains the most effective approach for the prevention of periodontal diseases. Several studies of the Disease Prevention and Health Promotion Branch address the prevention of periodontal diseases: 1. an analysis of National Health Interview Survey data to explore the knowledge of US adults regarding the signs and symptoms of gum disease to establish baseline information from which appropriate and targeted educational campaigns may be developed; 2. a study of two different school-based approaches to prevent gingivitis in teenagers; and 3) analysis of NHANES III data.

The 1990 Health Promotion Supplement to the National Health Interview Survey included a section on oral health and was administered to 41,104 respondents ages 18 years and older. Data were analyzed to compare the respondents knowledge of signs and symptoms of gum disease by selected sociodemographic and dental variables which included education, race, ethnicity, dental visit history, dentate status. Findings have been presented and a manuscript has been published.

A two year study of teenagers was conducted in York County, Virginia to determine the effectiveness of a self-assessment of gingival bleeding approach to the prevention of gingivitis compared with a plaque control approach. Both the plaque control and self-assessment of bleeding groups received an interactive manual describing the procedures they were to perform, classroom-based instruction and individual instruction specific to their needs. The individual instruction was reinforced following a 12 month interim oral examination. Findings show that while the two approaches did not differ, there were improvements in the gingival health status of both groups improved with over a 50% reduction in the mean number of gingival bleeding sites for both groups. A manuscript has been accepted for publication.

Ongoing consultation and assistance is provided for the planning and development of research conferences, symposia and research groups that focus on research related to periodontal disease prevention and oral health status improvement. Presentations are made as a part of science transfer efforts.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00596-02 DPHP

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Orofacial Pain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Drury, Thomas	Deputy Branch Chief, DPHP	EODDP, NIDR
Robinson, Dina	Health Promotion Research Specialist	EODPP, NIDR
Lipton, James	Spec. Asst. Train. & Career Development	EP, NIDR

COOPERATING UNITS (if any)

Other NIH Institutes; expert consultants

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

INSTITUTE AND LOCATION

NIDR,NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.45

PROFESSIONAL:

.45

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☒ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Data analysis and interpretation of orofacial pain data on existing surveys continue.

Section 1907 of the National Institutes of Health Revitalization Act of 1993 has requested that the Director of the National Institutes of Health (NIH), acting through the Director of the National Institute of Dental Research (NIDR) and through the heads of other NIH agencies, conduct a study of the frequency and health care costs of chronic pain conditions in the United States. The results of this study are to be submitted in a Report to Congress. The focus of this study is on the following selected chronic pain conditions: (1) chronic low back pain, (2) reflex sympathetic dystrophy RSD syndrome, (3) temporomandibular (TM) joint and muscle disorders, (4) posttherapeutic neuropathy, (5) painful diabetic neuropathy, (6) phantom pain, (7) post-stroke pain. The objectives of this study involve four evaluations: (1) of existing classifications, case definitions, diagnostic criteria, and case ascertainment procedures in the context of the phenomenology and clinical spectrum of these conditions and associated health care utilization, (2) of what is known about the direct medical care costs of these conditions, and (3) of the current state of the epidemiologic and economic science of chronic pain from the perspectives of basic, clinical, population, health services, and economic research. To achieve these objectives a series of background papers and reviews has been commissioned. These papers and reviews will be discussed at a Workshop in the Fall of 1995 in the Greater Washington, D.C. area. An analytical summary of these papers will provide the basis of the Report to Congress. The conduct and direction of this study will continue through FY 1995. To develop this Report to Congress a series of background papers and reviews have been commissioned. These papers and reviews will be discussed at a Workshop during 1995 in the Greater Washington, D.C. area. An analytical summary of these papers will provide the basis of the Report to Congress which is to be submitted to the Congress approximately two years from the signing of the 1993 Act.







DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00597-02 DPHP

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Healthy People 2000

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Gift, Helen C.	Chief, DPHP Branch	EODPP, NIDR
Horowitz, Alice M.	Education Specialist	EODPP, NIDR
Oldakowski, Richard	Chief, SPU, ASHAB	EODPP, NIDR
Small, John S.	Public Health Advisor	EODPP, NIDR
Nowjack-Raymer, R.	Public Health Research Spec.	EODPP, NIDR

COOPERATING UNITS (if any)

Centers for Disease Control  
Chief Dental Office

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Health Promotion Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.43

PROFESSIONAL:

.33

OTHER:

.10

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Oral health is a primary focus of Healthy People 2000, the nation's objectives for the 1990's. NIH is the co-lead agency for the oral health objectives and in that role assumes a responsibility for research to 1) monitor progress and 2) establish relevant programs to achieve the objectives. Staff of DPHPB serve on working groups, prepare reports and represent NIH on all Healthy People 2000 activities. All reports to the Assistant Secretary require analyses of existing data and reviews of literature to assure scientifically based responsiveness. Key topic areas this year have been use of fluorides, dental sealants, dental visits, nursing homes, orofacial trauma use of orofacial devices in organized sports, and HIV/AIDS prevention and control. In an effort to extend science transfer to the broader community, staff have developed papers related to objectives in Healthy People 2000 for publication in professional journals, and have worked with NCHS in the development of measures for upcoming survey which will improve assessment of the objectives.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00613-01 DPHP

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

NHANES III-PHASE I Analyses: Coordination and Team Leadership and Participation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Drury, Thomas	Deputy Branch Chief, DPHPB	EODPP, NIDR
Gift, Helen C.	Chief, DPHPB	EODPP, NIDR
Nowjack-Raymer R.	Public Health Research Specialist	EODPP, NIDR
Selwitz, Robert H.	Research Dentist	EODPP, NIDR
Horowitz, Alice M.	Education Specialist	EODPP, NIDR
Redford, Maryann	Public Health Specialist	EODPP, NIDR
Robinson, Dina	Health Promotion Research Specialist	EODPP, NIDR

COOPERATING UNITS (if any)

National Center for Health Statistics

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

INSTITUTE AND LOCATION

NIH, NIDR, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.89

PROFESSIONAL:

.84

OTHER:

.05

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

During the past 12 months, DPHP staff have been coordinating the activities of the Program dealing with the analysis of the oral examination data from the first three years of the 1988-1994 National Health and Nutrition Examination Survey (NHANES III-Phase I). Because of the many facets of oral health which were measured in NHANES III, analysis teams were formed to develop analytical and publication plans for subsets of the oral health data. Meetings were held on a regular basis to discuss preliminary abstracts of proposed papers, formal analysis plans, overlapping analytical issues, venues for the presentation of key findings, and publication vehicles. They have also provided a forum in which ideas on analytical topics were discussed. A series of descriptive papers are being prepared for publication as a special issue of a mainstream dental journal.

In addition to these overall coordinating activities, DPHP staff have also acted as team leaders for the analysis teams dealing with tooth loss, edentulism, quality of prostheses, and orofacial trauma. DPHP staff have also participated in the analysis teams for dental caries, periodontal diseases, soft tissue lesions, occlusal characteristics, tooth conditions and restorative treatment needs, global oral health indicators, and survey methodology. Participation in these groups has included the preparation and review of team abstracts, review of preliminary data runs, evaluation of data quality, development of summary measurements, analysis of preliminary tabulations, the development of paper outlines, and the preparation of presentations and papers for publication.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00617-01 DPHP

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Oral Manifestations of HIV in Women

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Redford, Maryann

Public Health Specialist

EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Health Promotion

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.55

PROFESSIONAL:

.50

OTHER:

.05

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Seemingly little attention had been paid to human immunodeficiency virus (HIV) infection in women. Much of what is currently known about HIV disease has been learned through the prospective study of large cohorts of gay men. Investigative efforts in the dental community have paralleled those in the general medical field. Most reports appearing in the dental scientific literature regarding the oral manifestations of HIV disease in women do not discuss how they relate to hormonal status and correlate with genital manifestations.

Recognizing that the multisite Women's Interagency HIV Study (WIHS) by the NIAID is poised to break scientific ground relative to HIV infection in women, NIDR branch staff have taken the leadership role in developing an oral component that includes data collection about the oral manifestations of HIV disease in women, particularly as they relate to hormonal status and correlate with genital manifestations. Significant participation of the NIDR in this collaborative effort provides an efficient, cost-effective and timely means targeting research towards several high-risk populations - women, minorities, and HIV infected individuals.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00622-01 MEDIB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Early Onset Periodontitis Gene Mapping

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Diehl, Scott R.	Chief, MEDIB	EODPP, NIDR
Wang, Shengbiao	MGES, MEDIB Branch	EODPP, NIDR
Sun, Cathy	MGES, MEDIB Branch	EODPP, NIDR
Freas-Lutz, Diana	MGES, MEDIB Branch	EODPP, NIDR
Eskandari, Tara	MGES, MEDIB Branch	EODPP, NIDR
Gillanders, Elizabeth	MGES, MEDIB Branch	EODPP, NIDR
Gregg, Mary	SGES, MEDIB Branch	EODPP, NIDR
Walczak, Cindy	SGES, MEDIB Branch	EODPP, NIDR
Bock, Carla	SGES, MEDIB Branch	EODPP, NIDR

COOPERATING UNITS (if any)

Schenkein, H. Medical College of Virginia  
Lopez, N. Chile

LAB/BRANCH

Molecular Epidemiology and Disease Indicators Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

4.14

PROFESSIONAL:

4.14

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Previous studies suggested that genetic variation in the HLA region of chromosome 6p may influence susceptibility to early onset periodontitis (EOP). Results of segregation analyses support the possibility that risk of EOP may be due to a single major gene. We conducted linkage analyses to evaluate the hypothesis that a gene within the HLA region significantly contributes to risk of EOP. Fifty families, with two or more close relatives affected by EOP, were ascertained in Virginia, USA and Chile. DNA was extracted from blood and a highly polymorphic marker located within the HLA region (near the Tumor Necrosis Factor Beta locus) was typed using the polymerase chain reaction. Linkage analyses were performed using a dominant model of disease transmission which is most strongly supported by previous studies. For the dominant model, assuming that EOP is a homogeneous disorder, our results statistically exclude the hypothesis that a susceptibility gene lies within 10cM (approximately 10 million bases of approximately 0.5% of the human genome). Additional analyses are planned for alternative modes of disease gene transmission. Under the assumption that EOP may actually consist of several etiologically distinct diseases having very similar clinical presentations our data still provide no support for HLA region involvement. However, our data do not statistically exclude (LOD <02.0) hypotheses of disease locus heterogeneity including models where up to half of our families contain a gene located in the HLA region which confers susceptibility to EOP. This is due to the limited power of even our relatively large collection of families and the inherent difficulties of mapping genes for disorders that have complex and heterogeneous etiologies. Additional statistical analyses, recruitment of families, and typing of flanking DNA markers are planned to more conclusively address these issues with respect to the HLA region and other candidate locations in the human genome.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00623-01 MEDIB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cleft Lip and Palate Gene Mapping

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Diehl, Scott R.	Chief, MEDIB	EODPP, NIDR
Miller-Chisholm, Ann	Asst. To Chief, MEDIB	EODPP, NIDR
Wang, Shengbiao	MGES, MEDIB Branch	EODPP, NIDR
Freas-Lutz, Diana	MGES, MEDIB Branch	EODPP, NIDR
Gillanders, Elizabeth	MGES, MEDIB Branch	EODPP, NIDR
Gregg, Mary	SGES, MEDIB Branch	EODPP, NIDR
Walczak, Cindy	SGES, MEDIB Branch	EODPP, NIDR
Bock, Carla	SGES, MEDIB Branch	EODPP, NIDR

COOPERATING UNITS (if any)

Erickson, R. University of Arizona  
Ballew, Carol CDC  
Mazaheri, M. Lancaster

LAB/BRANCH

Molecular Epidemiology and Disease Indicators Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.95

PROFESSIONAL:

.95

OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

Clefting of the lip and palate (CLP) is one of the most common craniofacial anomalies, with a rate of 1:650 in the United States population. Over 250 genetically based syndromes exist which include cleft lip or palate as a feature of their clinical presentation. In FY 1994, the NIDR initiated a collaborative research study with the Lancaster Cleft Palate Clinic in Lancaster. The Lancaster Collaborative Study of Cleft Lip and Palate (LCS) is a large scale epidemiologic study, involving the comprehensive mapping of highly informative DNA markers, utilizing the clinical population from the Lancaster Cleft Palate Clinic and affiliated clinics. Semiautomatic gene mapping technologies developed by MEDIB in ongoing CLP studies will be applied to map gene/genes responsible for clefting. The study population will consist of approximately 2300 patients and controls including 225 families with more than one child affected with CLP. DNA extracted from blood or buccal cells will be genotyped utilizing 29 panels of 12-17 microsatellite loci and genetic linkage and association analyses will be performed.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00624-01 MEDIB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Kartagener Syndrome Gene Mapping

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Diehl, Scott R.	Chief, MEDIB	EODPP, NIDR
Miller-Chisholm, Ann	Asst. to Chief MEDIB Branch	EODPP, NIDR
Wang, Shengbiao,	MGES, MEDIB Branch	EODPP, NIDR
Freas-Lutz, Diana	MGES, MEDIB Branch	EODPP, NIDR
Gillanders, Elizabeth	MGES, MEDIB Branch	EODPP, NIDR
Gregg, Mary	SGES, MEDIB Branch	EODPP, NIDR
Walczak, Cindy	SGES, MEDIB Branch	EODPP, NIDR
Bock, Carla	SGES, MEDIB Branch	EODPP, NIDR

COOPERATING UNITS (if any)

Witt, M. Poznan, Poland

LAB/BRANCH

Molecular Epidemiology and Disease Indicators Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.35

PROFESSIONAL:

1.35

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The immotile cilia syndrome (ICS) is a genetically determined disorder characterized by dysmotility or immotility of the cilia in airway epithelial cells, spermatozoa and other ciliated cells of the body. Kartagener syndrome (KS) is a subgroup of ICS characterized by a classic triad of symptoms: situs inversus, bronchiectasis and chronic sinusitis. Ciliary immotility is caused by various ultrastructural defects of cilia, predominantly by a lack of dynein arms. The clinical consequences of KS include pronounced craniofacial manifestations.

In FY94 a large scale collaborative genetic epidemiology study of KS was planned with Dr. Michael Witt in Poznan, Poland.

Over the next 12 months sixty Polish families with at least one child affected with KS will be recruited for this study. Coded DNA samples and research medical records will be sent to MEDIB, NIDR, NIH laboratory for genotyping.

To facilitate large scale genetic mapping of the human genome we will apply microsatellite markers suitable for use with a fluorescence-based automated DNA fragment analyzer. They are arranged into 29 sets, covering 22 autosome and the X chromosome, with an average interval of 10cM. Each set consists of 12-17 marker loci, with allele size ranges that do not overlap. Marker loci were selected on the basis of their reliability in PCR, polymorphism content, map position and the accuracy with which alleles can be scored automatically by the Genotypes™ program.

Classic pair wise linkage analyses using the program MENDEL which calculates the LOD score for complex diseases will be conducted in MEDIB. Multipoint linkage analyses will also be performed by calculating location scores using the programs LINKAGE and MENDEL.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00625-01 MEDIB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Etiology of Oral Cancer

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Schwartz, Joel L.

BDMS, MEDIB Branch

EODPP, NIDR

Mirth, Dale

BDMS, MEDIB Branch

EODPP, NIDR

COOPERATING UNITS (if any)

West, K.

Harvard School of Dental Medicine

Shklar, G.

Harvard School of Dental Medicine

LAB/BRANCH

Molecular Epidemiology and Disease Indicators Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.40

PROFESSIONAL:

.40

OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Lichen planus and oral leukoplakia, white lesions of the oral cavity were examined using immunohistochemical methods. The monoclonal antibodies and enzymes disclosed a dysregulation of cell growth not previously recognized in these possibly premalignant lesions. In the specific entity of erosive lichen planus we observed an abnormal expression for the p53, and the stress proteins in the oral mucosa. In the characteristic inflammatory infiltrate we noted an increase in Bcl-2 expression. Nucleosome formation was also found to be increased in the basal segment of the oral mucosa. Oral leukoplakia exhibiting dysplasia also showed high levels of p53 expression with localized areas of nucleosome formation, and Bcl-2 staining. Taken together these results indicated a profound dysregulation of cell growth and death.

Using tissues sections from a hamster buccal pouch oral mucosa treated with the carcinogen 7,12 dimethylbenz(a)anthracene (DMBA) a similar immunohistochemical study was performed using the identical monoclonal antibodies and enzymes. Early in the process of oral carcinogenesis we saw the expression of p53. and the stress proteins (70,25kD). Nucleosome formation was seen but was gradually replaced when carcinoma-in-situ was histopathologically evident, Bcl-2 was then noted in localized and expanding areas of the mucosa. In combination with markers for cell proliferation (PCNA, cell cycle) these studies indicated that oral carcinogenesis involved a suppression of programmed cell death and the normal expression of proteins such as p53 and stress proteins.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00626-01 MEDIB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Gene Mapping Panels

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Diehl, Scott R.	Chief, MEDIB Branch	EODPP, NIDR
Wang, Shengbiao	MGES, MEDIB Branch	EODPP, NIDR
Sun, Cathy	MGES, MEDIB Branch	EODPP, NIDR
Freas-Lutz, Diana	MGES, MEDIB Branch	EODPP, NIDR
Eskandari, Tara	MGES, MEDIB Branch	EODPP, NIDR
Gillanders, Elizabeth	MGES, MEDIB Branch	EODPP, NIDR
Gregg, Mary	SGES, MEDIB Branch	EODPP, NIDR
Walczak, Cindy	SGES, MEDIB Branch	EODPP, NIDR
Bock, Carla	SGES, MEDIB Branch	EODPP, NIDR

COOPERATING UNITS (if any)

Madden, D	Applied Biosystems Div. of Perkin Elmer
Budiansky, M.	Applied Biosystems Div. of Perkin Elmer
Gilbert, D.	Applied Biosystems Div. of Perkin Elmer

LAB/BRANCH

Molecular Epidemiology and Disease Indicators Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.22

PROFESSIONAL:

1.22

OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

We are evaluating 29 panels of fluorescently labeled markers located at approximately 10cM intervals. Each chromosome is covered at this marker density in 1-4 panels (11-17 loci/panel). Individual markers are labeled with 1 of 3 different fluorescent dyes, combined after PCR and run in a single gel lane. Genotypes are obtained for each locus using Applied Biosystems automated DNA Sequencer, and GENESCAN analysis, Genotypes, and Excel software. These programs automate the identification of alleles by distinguishing major peaks from PCR artifacts and facilitate the export of data in a format suitable for standard genetic analysis programs. To verify the reported genetic relationships among individuals involved in gene mapping studies, we developed software to determine the number of alleles shared among individuals within a family. We use these statistics to distinguish full and half sibs and parent-child relations from unrelated individuals. Finally, we are developing a database using Fourth Dimension software so that the tremendous amounts of data generated can be processed efficiently in an integrated suite of specialized computer programs for linkage/association studies.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 DE00627-01 MEDIB						
PERIOD COVERED October 1, 1993 to September 30, 1994								
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Allele Sharing Algorithms								
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">Diehl, Scott R.</td> <td style="width: 33%;">Chief, MEDIB Branch</td> <td style="width: 33%;">EODPP, NIDR</td> </tr> <tr> <td>Walczak, Cindy</td> <td>SGES, MEDIB Branch</td> <td>EODPP, NIDR</td> </tr> </table>			Diehl, Scott R.	Chief, MEDIB Branch	EODPP, NIDR	Walczak, Cindy	SGES, MEDIB Branch	EODPP, NIDR
Diehl, Scott R.	Chief, MEDIB Branch	EODPP, NIDR						
Walczak, Cindy	SGES, MEDIB Branch	EODPP, NIDR						
COOPERATING UNITS (if any) Dean, M.                      NCI								
LAB/BRANCH Molecular Epidemiology and Disease Indicators Branch								
SECTION								
INSTITUTE AND LOCATION NIDR, NIH, Bethesda, Maryland 20892								
TOTAL STAFF YEARS: .2	PROFESSIONAL: .2	OTHER:						
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews								
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>A computer algorithm is being developed to count the number of alleles that siblings and half siblings have in common, after the mother's alleles are subtracted from each child. These allele counts are averaged for each pair of people in question. Deviations from expected average values give an indication of problems in the pedigree structure (e.g., non-paternity, mislabeled DNA samples) when a large number of genetic markers are tested. The method has been used to examine 60 JP pedigrees with favorable preliminary results. When conducting linkage analysis studies using family pedigrees, it is important that the family structure is accurately reported, in order to detect linkage between an inherited disease and genetic marker. To improve our ability to verify family structures, we have developed a new strategy to evaluate allele sharing among family members.</p>								



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00628-01 MEDIB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Chemoprevention in Oral Cancer

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Schwartz, Joel L.

BDMS, MEDIB Branch

EODPP, NIDR

Mirth, Dale

BDMS, MEDIB Branch

EODPP, NIDR

COOPERATING UNITS (if any)

Shklar, G. Harvard School of Dental Medicine

LAB/BRANCH

Molecular Epidemiology and Disease Indicators Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.30

PROFESSIONAL:

.30

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Several studies were conducted to evaluate the role of chemopreventatives during oral carcinogenesis in the hamster buccal pouch tumor model. Tissue sections and cells which were treated in vivo were analyzed using immunohistochemistry, flow cytometry, and Western immunoblotting. Treatment with the carotenoid,  $\beta$ -carotene was administered to the hamsters producing fewer and smaller oral squamous cell carcinomas. The histopathology sections showed fewer areas of dysplasia and less invasive oral carcinomas once they did form. Results showed that nucleosome formation persisted from early to late carcinogenesis following treatment with  $\beta$ -carotene. Levels of Bcl-2 expression in contrast was not elevated during carcinogenesis with chemopreventative treatment. Stress proteins(70, 90) were increased very early during carcinogenesis while p53 was reduced in expression. Cell cycle was altered with the majority of the cells in G1, and showing less expression of PCNA. Treatment with either vitamin E or reduced glutathione also produced similar results. Transforming growth factor alpha and epidermal growth factor receptor expression was also noted to be reduced in staining while transforming growth factor beta was seen to be increased. Neovascularization was also apparently reduced as distinguished by fewer factor VIII antigen endothelial vascular spaces observed. These studies indicated that chemopreventative agents inhibited oral carcinogenesis by inducing programmed cell death, reducing growth factor expression, and inhibiting the development of angiogenesis. These results could lead to the development of new markers for early neoplasia identification in clinical tissues.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00629-01 MEDIB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Database for Genetic Epidemiological Studies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Diehl, Scott R.	Chief, MEDIB Branch	EODPP, NIDR
Walczak, Cindy	SGES, MEDIB Branch	EODPP, NIDR
Bock, Carla	SGES, MEDIB Branch	EODPP, NIDR

COOPERATING UNITS (if any)

Tiller, G                      4th Dimension Developer

LAB/BRANCH

Molecular Epidemiology and Disease Indicators Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.25

PROFESSIONAL:

.25

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects   ☐ (b) Human tissues   ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

MEDIB has begun developing, implementing and documenting a comprehensive laboratory data management system. This system will be used for the management of clinical, pedigree and genotype data for gene mapping studies. The database format shall be used for multiple databases (among them, juvenile periodontitis, cleft lip and palate and Kartagener Syndrome). The databases and programs will be implemented in 4th Dimension (4D) on Mackintosh computers. Mr. George Tiller, 4D Register Developer, is cooperating with MEDIB staff to develop appropriate procedures and documentation. The completed system will integrate and manage clinical information, family histories, and marker-allele typing as well as provide interactive procedures for 1) extensive error checking 2) generation of formatted files suitable for input to various linkage analysis programs, and 3) provide primer inventories and for tracking DNA samples and the cell lines produced from them.

Future effects will include developing procedures for 1) checking genetic incompatibilities and 2) rounding and banning allele sizes for more direct input into the database.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00630-01 MEDIB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Development of Analytical Programs for Genetic Epidemiological Studies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Diehl, Scott R.	Chief, MEDIB Branch	EODPP, NIDR
Walczak, Cindy	SGES, MEDIB Branch	EODPP, NIDR
Bock, Carla	SGES, MEDIB Branch	EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Molecular Epidemiology and Disease Indicators Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.20

PROFESSIONAL:

.20

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects   ☐ (b) Human tissues   ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Gene mapping studies using DNA markers to detect craniofacial and oral diseases utilize a wide variety of statistical techniques. We are developing a scheme to better integrate these software tools. Since these programs were written elsewhere using a variety of computer languages and input for integrating them with our interval database will streamline the analysis process. The programs include:

- 1) MENDEL, to calculate log odds and estimate linkage between disease and genetic DNA marker loci,
- 2) affected sibling pair method
- 3) affected pedigree marker method
- 4) CRI-MAP to determine multi-point marker
- 5) HETERGEN - to test for linkage allowing for possible locus heterogeneity and calculating admixture odds LOD scores
- 6) SIMLINK - to estimate the probability or power of detecting linkage for a set of family pedigrees. This program simulates cosegregation of trait and marker loci in pedigrees resulting in an objective power calculation to determine if the collection of families is sufficient to demonstrate linkage.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00631-01 MEDIB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular and Epidemiological Studies of Waardenburg Syndrome

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Diehl, Scott R.

Chief, MEDIB Branch

EODPP, NIDR

Bock, Carla

SGES, MEDIB Branch

EODPP, NIDR

COOPERATING UNITS (if any)

Nance, W.

Medical College of Virginia

Arnoe, K.

Gallaudet

LAB/BRANCH

Molecular Epidemiology and Disease Indicators Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.20

PROFESSIONAL:

.20

OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We performed linkage analyses and tests of locus heterogeneity of Waardenburg Syndrome (WS) using 9 DNA markers from 2q35-37, including two highly polymorphic microsatellites very closely linked to the candidate PAX3 locus. Analysis of 14 WS Type 1 (WS1) families at PAX3 yielded a maximum LOD score of 27.81,  $\theta_f = .010$ ,  $\theta_m = .007$  assuming homogeneity. However, we found significant evidence of heterogeneity in our study, with approximately 90% of our families linked to the PAX3 region. None of five WS Type 2 (WS2) families showed linkage to the PAX3 candidate region, and linkage was excluded ( $\text{LOD} < -2.0$ ) up to a distance of 17.5 cM. We localized the marker D2S102 to less than 1cM from PAX3 locus ( $\theta = 0$ ), and thus were unable to determine whether it mapped distally or proximally due to lack of crossovers between these two markers. Meiotic breakpoint analysis in one of the three families with a crossover between WE1 and PAX3 provides strong evidence that the disease gene in this family is located elsewhere in the genome.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00632-01 MEDIB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular and Epidemiological Studies of Schizophrenia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Diehl, Scott R.	Chief, MEDIB Branch	EODPP, NIDR
Wang, Shengbiao	MGES, MEDIB Branch	EODPP, NIDR
Sun, Cathy	MGES, MEDIB Branch	EODPP, NIDR
Gregg, Mary	SGES, MEDIB Branch	EODPP, NIDR
Walczak, Cindy	SGES, MEDIB Branch	EODPP, NIDR
Bock, Carla	SGES, MEDIB Branch	EODPP, NIDR

COOPERATING UNITS (if any)

Gersher, E.	NIMH
Golden, L.	NIMH
Byerley, W.	University of Utah

LAB/BRANCH

Molecular Epidemiology and Disease Indicators Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.00

PROFESSIONAL:

1.00

OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Extensive genome scanning techniques using automated fluorescent microsatellite typing methods in combination with linkage analysis have been used to demonstrate evidence of linkage to Schizophrenia on chromosome 6pter-p22. The collaborative findings based on analysis of 186 multiplex Schizophrenia families have been submitted for publication. Genome scanning techniques from this collaborative effort are being applied to ongoing studies of early onset periodontitis and cleft lip and palate.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE006333-01 MEDIB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Quantitative Trait Loci (QTL) Mapping of Cleft Lip and Palate

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Diehl, Scott R.

Chief, MEDIB Branch

EODPP, NIDR

Bock, Carla

SGES, MEDIB Branch

EODPP, NIDR

COOPERATING UNITS (if any)

Erickson, R. University of Arizona

LAB/BRANCH

Molecular Epidemiology and Disease Indicators Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.09

PROFESSIONAL:

.09

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The human/mouse homology map provides an excellent tool to identify candidate genes involved in human disease. Studies have been initiated using several newly identified loci in man that are candidates for involvement in facial clefting. In collaboration with Dr. Erikson, we have used markers for homologous positions in the mouse to search for QTLs affecting facial clefting. The search identified a region of mouse chromosome 3 homologous to human 1q21 as increasing liability to sporadic CL(P) when the A/J strain allele is present. This effect might be expected since the A/J strain has much higher incidence of sporadic CL9P) than does the C57Bl6J strain, the other progenitor strain of the RI lines.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00635-01 MEDIB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies with Odontogenic Tumors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Schwartz, Joel L.

BDMS, MEDIB

EODPP, NIDR

Mirth, D.

BDMS, MEDIB

EODPP, NIDR

COOPERATING UNITS (if any)

Miller, G.

Navy Dental Research

Kratochvil, F.

Chairman Navy Dental Oral Pathology

LAB/BRANCH

Molecular Epidemiology and Disease Indicators Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.30

PROFESSIONAL:

.30

OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This laboratory has established a series of odontogenic cell lines. These cell lines are primary cell lines and include at least one of each of the following lesions: 1) ameloblastoma, 2) odontogenic fibroma, 3) myxoma, 4) keratinizing odontogenic cyst, 5) gingival fibromatosis and normal gingival fibroblasts and oral mucosa from the identical patient, and 6) normal gingival fibroblasts and periodontal ligament fibroblasts.

The cell lines have been examined by a scanning and ultrastructural electronmicroscopy. The ameloblastoma, myxoma, gingival fibroblastosis, and odontogenic fibroma have produced lesions in tumorigenicity assays with the nude mouse.

The odontogenic fibroma has also been analyzed for its protein profile, which is significantly different from the protein profiles of normal gingival fibroblasts or periodontal ligament fibroblasts. Western immunoblotting further demonstrated decreased levels of expression for minimum, chondrotin sulfate, and N-CAM. Laminin was also decreased in expression following treatment with increased amounts of fibroblasts growth factors. Differences between the various fibroblast populations and collagen expressions, as well kinase activities were also observed to be altered. For example, the level of protein kinase C was elevated in the fibroma derived fibroblasts compared to the other populations. Treatment of the fibroma derived fibroblasts with fibroblast growth factor beta also resulted in an high level of expression for IL-1 beta, and proliferation. This laboratories further investigating a relationship between growth factor response and lymphokine production by these cell lines. These studies could provide a control of cell growth in the jaw reducing bone damage.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 DE00636-01 MEDIB
PERIOD COVERED October 1, 1993 to September 30, 1994		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Clinical Biomarkers Studies		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Schwartz, Joel L.                      BDMS, MEDIB                      EODPP, NIDR Diehl, Scott R.                      Chief, MEDIB Branch              EODPP, NIDR		
COOPERATING UNITS (if any) Kramer, A.                      University of San Francisco Lehman, T.                      BioServe Biotech. Ltd.		
LAB/BRANCH Molecular Epidemiology and Disease Indicators Branch		
SECTION		
INSTITUTE AND LOCATION NIDR, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: .16	PROFESSIONAL: .16	OTHER:
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  This laboratory is developing a number of clinical trials investigating the diagnosis, etiology, prevention and treatment of oral malignant disease. The first trial will involve a population obtained from the veterans administration. Oral lesions suspected of having the potential to develop into malignancy will be removed and analyzed for various indicator markers of oral transformation. These include markers that specify changes in programmed cell death, dysregulation of cell growth and proliferation and response to growth factors such as epidermal growth factor related proteins. The second study will use a population at high risk to develop oral cancer because they have developed papillary verrucous leukoplakia. The above general categories of markers will also be used to identify premalignant changes, but we will also examine the DNA fingerprints from these patients normal, premalignant, and cancerous tissue. A synthetic retinoid will be used to treat patients in an randomized fashion. The efficacy of this agent to change the pattern for oral transformation will be observed in association with these above genetic and immunohistochemical analyses. A third smaller study will be conducted to further enhance our techniques in developing genetic fingerprinting of oral premalignant and malignant tissue.		



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 DE00637-01 MEDIB
PERIOD COVERED October 1, 1993 to September 30, 1994		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Experimental Chemoprevention In Vitro		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Schwartz, Joel L.                      BDMS, MEDIB                      EODPP, NIDR		
COOPERATING UNITS (if any)		
LAB/BRANCH Molecular Epidemiology and Disease Indicators Branch		
SECTION		
INSTITUTE AND LOCATION NIDR, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: .15	PROFESSIONAL: .15	OTHER:
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  Chemopreventative agents such as retinoid, carotenoid, tocopherol, and glutathione were used in treatments of human and hamster oral carcinoma cells. Immunohistochemistry, Western immunoblotting, in situ hybridization, and flow cytometry was used to detect biochemical changes associated with changes in tumor cell growth. The carotenoid b-carotene was found in a dose response manner to induce DNA fragmentation, inhibiting calcium flux, and reducing non protein sulfhydryl (glutathione) and glutathione-S-transferase. The protein expression pattern also confirmed an enhancement of programmed cell death. P53 wild type and stress proteins (70,90kD), were increased in expression. Mutant p53 and Bcl-2 were also reduced in expression. Cell cycle analysis showed an accumulation of the tumor cells in G1 of the cell cycle. The cycling A and D were also increased in expression as well as kineses, cdc2 and cdcHs were found to be enhanced in expression. Protooncogene expression for c-neu, and/or erbB2 was noted to be depressed. The other chemopreventative agents produced in general similar responses in the oral cnace cells. The results indicated that chemopreventative agents could suppress the growth of human and hamster cancer cells in a selective manner by inducing programmed cell death in the tumor cells. These studies may help in the development of new therapeutic approaches for the control of tumor cell growth.		





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER Z01 DE00638-01 MEDIB
PERIOD COVERED October 1, 1993 to September 30, 1994		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Isolation and Characterization of Odontogenic Myxoma		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Schwartz, Joel L.                      BDMS, MEDIB                      EODPP, NIDR		
COOPERATING UNITS (if any) Wright, L.                      Tugaloo, Mississippi Kratochvil, F.                      Navy Dental Oral Pathology		
LAB/BRANCH Molecular Epidemiology and Disease Indicators Branch		
SECTION		
INSTITUTE AND LOCATION NIDR, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: .15	PROFESSIONAL: .15	OTHER:
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>             An odontogenic myxoma was placed into cell culture. We obtained two cell lines, one which appeared fibroblast like, the other more epidermal. The more epidermal cell line was observed to have a high doubling time, grew in an anchorage independent manner, and developed tumor in nude mice. Ultrastructure analysis indicated that some of these cells were myofibroblasts, even though they developed into an adenocarcinoma like lesion in the nude mouse. They increased in growth in response to fibroblast growth factor. Immunohistochemistry demonstrated increased expression of the transcription factors, c-fos c-jun (AP-1, heterodimer complex), and c-myc. Cell cyclin proteins were also increased in expression. Apoptosis related proteins were not highly expressed such as p53, or the stress proteins. Bcl-2 was found to be present in these cells, as were the epidermal growth factor receptor, and the protooncogene c-neu. Further studies identifying the genetic relationship between these cells and the original patient biopsy are under way. These studies may provide a deeper understanding for the medical control of these aggressive oral tumors.           </p>		



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00497-06 OD

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Forecasting Dental Health and Utilization Using A Microsimulation Model

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Brown, L. Jackson

Director

EODPP, NIDR

Zion, Gary R.

Computer Programmer, HAS, ASHAB

EODPP, NIDR

Oldakowski, Richard J.

Chief, SPU, ASHAB

EODPP, NIDR

COOPERATING UNITS (if any)

Cornell University, Department of Sociology, Ithica, New York and the University of Michigan, Ann Arbor, Michigan

LAB/BRANCH

Analytical Studies and Health Assessment Branch/Office of the Director, EODPP

SECTION

Health Assessment Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.44

PROFESSIONAL:

.44

OTHER:

.00

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A computer model which will generate condition forecasts of future tooth loss, dental status, service utilization and expenditures for individuals and families in the U.S. was developed under a contract with Cornell University. The forecasts will be developed in considerable sociodemographic detail. Several noted dental specialists and modeling experts were also consultants to the project. Microsimulation is the approach being used. The development of the model and the production of initial forecasts has been completed. Tests of the full model are being conducted by EODPP staff. The model has been adapted to NIH computer systems and resides on a RISC where new SAS software to analyze the output is being developed. Starting from a representative sample of persons and families, the NIDR micro model will forecast tooth loss, dental health conditions, and dental service use for persons identified by age, gender, race, education, income, and other putatively important explanatory variables. Policy experiments with the full model are planned both for past times and also for future times. As a framework for synthesizing research findings, the NIDR micro model will provide a vehicle for carrying out experiments in which the latest dental research can be applied consistently and systemically to key dental policy issues.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00570-03 OD

PERIOD COVERED

October 1, 1993 - September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Some Power Considerations When Deciding to Use Transformations

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Kingman, A. Chief Statistician, EODPP, NIDR  
Zion, G. Computer Programmer/Analyst, EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Epidemiology and Oral Disease Prevention Program

SECTION

Office of the Director

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.35

PROFESSIONAL:

.35

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Conventional wisdom suggests that one either perform a power transformation (log, square root, etc) to data derived from studies with small sample sizes whose response variables have non-normal distributions before analysis, or use a distribution-free procedure such as a rank transformation or a randomization test procedure. To better appreciate the effect of specific alternatives on both the type I error and power of detecting differences between treatment groups, simulation studies were conducted assuming specific gamma distributions  $G(r, 0)$ . A simple two group design was assumed. The reference group always had an average disease level  $\mu = r \cdot 0 = 3.0$ , and the treatment group always had means whose percentage reductions ranged from 0% to 50%. By varying the shape parameter  $r$  from 1, 2, 4, 8, 16 one could investigate distributional profiles having almost symmetric distributions ( $r = 16$ ) to those with highly skewed distributions ( $r = 1$  or 2). Six statistical test procedures were compared. All test procedures were robust relative to the type I error. The UMP test based on a ratio of sample means produced the greatest power for all combinations of  $n$ ,  $r$  and  $r/R$ . The power losses associated with the randomization test, the  $t$ -test on original scale, and the  $t$ -test on the square root scale were very small, (3% to 6% in absolute value) for  $n = 10$  and 15, and less than 2% for group sizes of 25 or more. The power loss associated with the  $t$ -test on the log scale was larger, ranging from 5% to 10% smaller power than the  $t$ -test on original scale. The Wilcoxon rank test produced similar results to that of the LOG  $t$ -test for small samples. The loss in power for the unshifted LOG test could be recouped by use of a shifted LOG ( $x + c$ ) test. The same procedures based on differences in sample means were then compared to comparable lognormal distributions. Here the log transformation performed the best, better than the Wilcoxon rank test, and both considerably better than the  $t$ -test on the original scale. These results suggest that statistical inferences can be highly dependent on both the distributional form of the response variable and the scale of measurement used in the statistical analysis.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00582-02 OD

PERIOD COVERED

October 1, 1993 - September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Techniques for Measuring Dental Fluorosis; Issues in Data Analysis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Kingman, A. Chief Statistician, EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Epidemiology and Oral Disease Prevention Program

SECTION

Office of the Director

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.10

PROFESSIONAL:

.10

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The actual fluorosis prevalence value reported for a population can vary considerably among different scoring systems and within a scoring system due to intrinsic factors defining a case. Currently popular scoring systems evaluate the level of fluorosis are based on different measurement units, diverse numbers of sites per person, and distinctly different groupings of clinical symptoms. Intrinsic factors for a scoring system include the inclusion of a questionable category, together with both the level of fluorotic involvement and the number of sites within a subject required for case definition. None of these factors are related to the level of fluoride exposure in the examined population. Case definitions for each scoring system are desirable, essential for obtaining prevalence estimates, but currently not available for all scoring systems. Dean's scoring system has been the most widely used, includes a case definition, and thus, despite its peculiarities, will probably continue to play the role of reference standard. Ratios of fluorosis prevalence magnitudes, as evidenced by odds ratios, can be more stable between scoring systems for comparing groups having different fluoride exposure levels. There is a strong correlation among extent and specific measures of fluorosis severity for the DI and TSIF scoring systems, as well as within each scoring system separately. Parallel patterns in fluorosis severity were found among groups exposed to different levels of fluoride. The effects of fluoride exposure are best understood using relative measures contrasting severity levels of fluorosis for two or more fluoride exposure levels.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 DE00583-02 OD
PERIOD COVERED October 1, 1993 - September 30, 1994		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The NIDR Amalgam Study and Health Effects Study Protocol		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)  Kingman, A.                      Chief Statistician, EODPP Albertini, T.                  Special Asst. to Director, EODPP Brown, J.                      Director, EODPP		
COOPERATING UNITS (if any)  USAF AL/AEOP Brooks Air Force Base, TX		
LAB/BRANCH OD		
SECTION EODPP		
INSTITUTE AND LOCATION NIDR, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 0.55	PROFESSIONAL: 0.55	OTHER:
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>             This is a major study investigation whether there is any evidence of adverse health effects attributable to exposure to dental amalgams in a specific adult populations. The NIDR Amalgam Study involves 1166 Air Force Veterans, roughly representing a 50% subsample of the Air Force Health Study participants. These participants were examined for scores of medical conditions, including many which would potentially be affected by exposure to mercury. Dental Examinations and blood and urine samples were obtained from the 1166 participants who are in the NIDR Amalgam Study. The exact type of restorative material used in exposure was defined as the total number of surfaces having an amalgam restoration present. All soft tissue conditions detected were obtained. Mercury levels in blood and urine were recorded in µg/l.           </p> <p>             The amalgam exposure for this cohort was determined to be rather high, averaging 20 surfaces on average. The blood and urinary Hg levels were found to be low, averaging 2.9 µg/l and 3.1 µg/l, than 15 µg/l, but these levels could not be ascribed to dental amalgam exposure. There was a clear dose response association found between amalgam exposure and urinary Hg levels, but none found between amalgam exposure and blood Hg levels.           </p> <p>             The health data will be obtained from the AFHS scientists shortly and the tedious, complex analytical process begun.           </p>		



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00602-02 OD

PERIOD COVERED

October 1, 1993 - September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Effect of Missing Teeth in the Definition and Calculation of Caries Increments

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Kingman, Albert                      Chief Statistician,      EODPP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Epidemiology and Oral Disease Prevention Program

SECTION

Office of the Director

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.10

PROFESSIONAL:

.10

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects    ☐ (b) Human tissues    ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

When defining caries increments in children and young adults it is customary to refer to DMFS increments as net increment, NI(DMFS), and it is defined as the number of sound surfaces which become "decayed" (SD+SF+SM) minus the number of "decayed" surfaces that become sound (DS+FS+MS). If increment is defined as the difference in time-specific DMFS scores, ie.  $DIF(DMFS) = DMFS_2 - DMFS_1$ , then it is easily shown that  $NI(DMFS) = DIF(DMFS)$ .

Many investigators define increments using change in the DFS scores for older adult populations because the reason for missing teeth is often unknown. However, the consequence of ignoring missing teeth in the definition produces inconsistencies between increment estimates because missing surfaces behave both like sound and "decayed" surfaces. If one were to estimate DFS increment by NI(DFS), the analog of NI(DMFS), where  $NI(DFS) = (SD+SF) - (DS+FS)$ , it would no longer be equivalent to the analog  $DIF(DFS)$ , where  $DIF(DFS) = DFS_2 - DFS_1$  as one might expect. But rather the relationship between increment estimates now becomes  $DIF(DFS) = NI(DFS) + ("new\ lesions") - ("reversals")$ . The "new lesions" (MD and MF) and "reversals" (DM and FM) terms are a consequence of not accounting for missing surfaces. Even if the true cause of tooth loss could be determined for all missing teeth, neither increment estimate appropriately counts surfaces for which caries progresses, treated or untreated, to the point where an extraction is performed.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH  
SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00621-01 OD

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the  
Distribution of Dental Restorative Materials in a military population

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal

Albertini, Tullio F. Special Asst. for Program Mngt, EODPP, NIDR

Kingman, Albert NIDR Chief Statistician, EODPP, NIDR

Brown, L. Jackson Director, EODPP, NIDR

COOPERATING UNITS (if any)

United States Air Force, AL-AEOP

LAB/BRANCH

Epidemiology and Oral Disease Prevention Program

SECTION

Office of Director

INSTITUTE AND LOCATION

NIH, NIDR, Bethesda, Maryland

TOTAL STAFF YEARS:

.30

PROFESSIONAL:

.30

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☒ (b) Human ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

An important aspect of the NIDR Amalgam Study of Air Force Veterans is an inventory of dental restorative materials found in the study population which can serve as the basis for constructing exposure variables. This project reports on the distributions of dental materials found in a military population. Dental examinations were performed on 1166 male veterans to obtain tooth and surface specific data on existing restorations. Dental materials were classified into five categories: amalgams, resins, porcelains (including cements and temporaries), gold and other metals. The mean age of these veterans was 52.8 years. Overall, 5.2% of the participants were edentulous. Dentate individuals averaged 19.9 amalgam surfaces per person, 18.4 resin and porcelain surfaces combined and 10.4 surfaces for gold and other metals combined. Slightly more than 1/2 of all restored surfaces in dentate individuals were restored with amalgam. Unlike all other restorative materials whose mean number of restored surfaces increased with age, the number of amalgam surfaces varied with age peaking at 22.1 surfaces in the 50-54 year old age group to a low of 14.7 surfaces in the 5-78 year old age group.



<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		<b>PROJECT NUMBER</b> Z01 DE 00634-01 OD
<b>PERIOD COVERED</b> October 1, 1993 to September 30, 1994		
<b>TITLE OF PROJECT</b> (80 characters or less. Title must fit on one line between the <u>A Retrospective Analysis of Dental Manpower Supply Projections</u>		
<b>PRINCIPAL INVESTIGATOR</b> (List other professional personnel below the Principal Albertini, Tullio F.                      Special Asst. for Program Mngt, EODPP, NIDR Clark, Norman                              BHPR, HRSA Bronstein, Gloria                        BHPR, HRSA Bernstein, Stuart                        BHPR, HRSA		
<b>COOPERATING UNITS</b> (if any) Bureau of Health Profession, HRSA, PHS		
<b>LAB/BRANCH</b> Epidemiology and Oral Disease Prevention Program		
<b>SECTION</b> Office of Director		
<b>INSTITUTE AND LOCATION</b> NIH, NIDR, Bethesda, Maryland		
<b>TOTAL STAFF YEARS:</b> .15	<b>PROFESSIONAL:</b> .15	<b>OTHER:</b>
<b>CHECK APPROPRIATE BOX(ES)</b> <input type="checkbox"/> (a) Human <input type="checkbox"/> (b) Human <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
<b>SUMMARY OF WORK</b> (Use standard unreduced type. Do not exceed the space provided.) This study consists of a retrospective analysis of the accuracy of dentists supply projections contained in eight reports to the President and Congress on the supply of health professional in the U.S. This represents the first long term external validation of dentists federal supply projections. Since the reports are important sources of information for dental educational institutions, it is important to provide planners and researchers with an assessment of the accuracy of such federal estimates.		





<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		<b>PROJECT NUMBER</b>  Z01 DE 00639-01 OD
<b>PERIOD COVERED</b> October 1, 1993 to September 30, 1994		
<b>TITLE OF PROJECT</b> <i>(80 characters or less. Title must fit on one line between the NHANES III- Workgroup Participation)</i>		
<b>PRINCIPAL INVESTIGATOR</b> <i>(List other professional personnel below the Principal)</i> Brown, L. Jackson      Director, OD, EODPP, NIDR Kingman, Albert              NIDR Chief Statistician, OD, EODPP, NIDR Albertini, Tullio F.              Special Asst. for Program Mngt, EODPP, NIDR		
<b>COOPERATING UNITS</b> <i>(if any)</i>		
<b>LAB/BRANCH</b> Office of Director, EODPP		
<b>SECTION</b>		
<b>INSTITUTE AND LOCATION</b> NIH, NIDR, Bethesda, Maryland		
<b>TOTAL STAFF YEARS:</b> .35	<b>PROFESSIONAL:</b> .30	<b>OTHER:</b> .05
<b>CHECK APPROPRIATE BOX(ES)</b> <input checked="" type="checkbox"/> (a) Human <input type="checkbox"/> (b) Human <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
<b>SUMMARY OF WORK</b> <i>(Use standard unreduced type. Do not exceed the space provided.)</i> During the past Fiscal Year, OD staff have provided management oversight, professional advice and participated in the analysis of the oral examination data from the first phase of the National Health and Nutrition Examination Survey-III. This survey is a major source of national data on the prevalence of oral health conditions in the U.S. population. The staff participated in the development of analytical and publication workplans including periodontal disease, caries, tooth conditions and restorative treatment needs, and a number of other specific topical areas. Participation also included the preparation and review of abstracts, review of preliminary data runs, evaluation of data quality, development of summary measurements, analysis of tabulations, development of publication schedules and outlines, and the preparation of presentations and papers for dissemination.		



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00640-01 OD

PERIOD COVERED

October 1, 1993 - September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Associations Between Amalgam Exposure and Hg Levels in Urine and Blood

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Kingman, A. Chief Statistician, EODPP  
Albertini, T. Special Asst. to Director, EODPP  
Brown, J. Director, EODPP

COOPERATING UNITS (if any)

USAF AL/AEOP  
Brooks Air Force Base, TX

LAB/BRANCH

OD

SECTION

EODPP

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.15

PROFESSIONAL:

0.15

OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Dental Examinations and blood and urine samples were obtained from a sample of 1166 adult Air Force Personnel participating in the AFHS. The exact type of restorative material used in dental fillings was recorded for each affected surface. Amalgam exposure was defined as the total number of surfaces having an amalgam restoration present. Mercury levels in blood and urine were recorded in ug/l.

The amalgam exposure for this cohort was determined to be rather high, averaging 20 surfaces on average. The blood and urinary Hg levels were found to be low, averaging 2.9 ug/l and 3.1 ug/l, respectively. There were 11 participants who had aHg levels greater than 15 ug/l, but these levels could not be ascribed to dental amalgam exposure. There was a clear dose response association found between amalgam exposure and urinary Hg levels, but none found between amalgam exposure and blood Hg levels.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00641-01 OD

PERIOD COVERED

October 1, 1993 - September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Direct Versus Indirect Estimation of Amalgam Exposure in Adult Males

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Kingman, A. Chief Statistician, EODPP  
Albertini, T. Special Asst. to Director, EODPP  
Brown, J. Director, EODPP

COOPERATING UNITS (if any)

USAF AL/AEOP  
Brooks Air Force Base, TX

LAB/BRANCH

OD

SECTION

EODPP

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.03

PROFESSIONAL:

0.03

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Investigators have suggested that the effects of exposure to mercury vapor from dental amalgams be investigated by using data bases including various health outcomes measures and coronal caries DFS scores. In such data bases one is confronted with having to estimate amalgam exposure by an indirect method (for example, assume that all restorations on occlusal surfaces are amalgam). The effect of misclassification was investigated by using the NIDR Amalgam Study data base. In this study the coronal caries scores were obtained by the standard NIDR criteria used in epidemiological surveys. All restorative materials found in these study participants were also documented at the surface level. Thus, direct estimation of the total number of amalgam surfaces for each participant was possible as well.

The effect of various indirect estimates of amalgam exposure were estimated. The result of assuming that all occlusal restorations involved amalgam material produced positively biased estimates of amalgam exposure which ranged from 30% to 70% of their true values. The magnitude of the bias was not consistent over the age range 40 - 70 years of age.













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